ARYL IMIDAZOLES AND THEIR USE AS ANTI-CANCER AGENTS

FIELD OF INVENTION

This invention pertains to the field of anti-cancer compounds and, in particular, to the use of therapeutically active 2,4,5-trisubstituted imidazole compounds in the treatment of cancer.

BACKGROUND OF THE INVENTION

A cancer is a malignant tumour of potentially unlimited growth. It is primarily the pathogenic replication (a loss of normal regulatory control) of various given types of cells found in the human body. By select mutation resulting from a primary lesion, the DNA of a cancer cell evolves and converts the cell into an autonomous system. Conventional cancer treatments have focused mainly on killing cancerous cells. Chemotherapeutic agents currently used for anti-cancer/anti-tumour therapy are selected for their toxicity towards rapidly proliferating cells. Most of them cause undesirable systemic effects such as cardiac or renal toxicity, marrow aplasia, alopecia, nausea and vomiting. During the last few years, many researchers have tried to eliminate these side effects by developing drugs having suitable physico-chemical properties allowing an increase of the availability of the drug to the tumour site. New molecules extracted from natural sources, synthetically or semi-synthetically produced, enzymes, radioisotopes, DNA toxins, various macromolecules, and antibodies against fibrin or against tumour-specific surface antigens are bound to drugs in an attempt to increase selectivity of the chemotherapeutic agents.

The effectiveness of most anticancer agents is greatly reduced because of their high toxicity and the nature of the illness. It is believed that the problem of high toxicity of the anticancer agents can be circumvented by chemical modifications of those structures in such a way that they act more specifically on tumour cells without increasing systemic toxicity. The research in this field is therefore mainly directed to

the synthesis of anticancer agents which would possess high antineoplastic activity, low systemic toxicity and low mutagenicity on normal cells.

Heterocyclic compounds, especially heterocyclic azole derivatives, have been shown to have a wide spectrum of biological activities. One class of compounds with interesting biological activities is the imidazoles (derivatives containing a five-membered heterocyclic azole). A variety of biological activities have been reported for imidazole derivatives with different substitution patterns (Lee *et al. Nature* 1994 **327**:739-745; Abdel-Meguid *et al. Biochemistry*, 1994, **33**:11671; Heerding *et al. Bioorg. Med. Chem. Lett.* 2001, **11**:2061-2065; Bu *et al. Tetrahedron Lett.* 1996, **37**:7331-7334; Lewis JR. *Nat. Prod. Rep.* 1999, **16**:389-418; Lewis JR. *Nat. Prod. Rep.* 1998, **15**:417-437 and 371-395).

Biological activities have also been reported for aryl-imidazole derivatives, for example, these compounds can act as modulators of multi-drug resistance in cancer cells (Zhang *et al. Bioorg. Med. Chem. Lett.* 2000, **10**:2603-2605), inhibitors of p38 MAP kinase (Adams *et al. Bioorg. Med. Chem. Lett.* 2001, **11**:867-2870, McLay *et. al. Bioorg. Med. Chem.* 2001, **9**:537-554) and of cytokines (U.S. Patent Nos. 5,656,644; 5,686,455; 5,916,891; 5,945,418; and 6,268,370), and inhibitors of bacterial growth (Antolini *et al. Bioorg. Med. Chem. Lett.* 1999, **9**:1023-1028).

A few reports have indicated that triaryl-imidazole compounds can act as inhibitors of p38 MAP kinase (for example, see LoGrasso *et al. Biochemistry.* 1997, **36**:10422-10427) and as modulators of multi-drug resistance in cancer cells (Sarshar *et al. Bioorg. Med. Chem. Lett.* 2000, **10**:2599-2601), however, the majority of the literature indicates that these compounds have found use mainly as colour producing reagents (U.S. Patent Nos. 4,089,747; 5,024,935; 5,047,318; 5,496,702; 5,514,550; and 5,693,589) and as photopolymerization initiators (U.S. Patent Nos. 6,117,609 and 6,060,216), generally in dimeric form.

The potential anti-cancer activity of a number of compounds has been investigated by the National Cancer Institute (NCI), which has undertaken a large scale screening of several thousand compounds to try to identify those that have potential therapeutic application in the treatment of cancer (NCI Yeast Anticancer Drug Screen). The

screen is based on the ability of candidate compounds to inhibit the growth of *Saccharmyces cerevisiae* strains that have mutations in genes related to cell cycle control and DNA repair damage. Compounds are initially screened against a panel of six yeast strains at a single concentration (Stage0). Compounds with activity in Stage0 are re-screened against the same panel at two concentrations (Stage1). Selected compounds with activity in Stage1 that also show selectivity are re-screened against a panel of 13 yeast strains at five concentrations (Stage2). Many of the results from the screening have been made available on the NCI/DTP website. The approach adopted in this screen is dependent on a candidate compound exerting its activity on certain cellular pathways (*i.e.* cell cycle control or DNA repair damage). The results generated by this type of screen, therefore, represent a very preliminary stage of screening for potential anti-cancer drugs and do not necessarily correlate with the ability of a compound to inhibit the growth of cancer cells *in vitro* or *in vivo*.

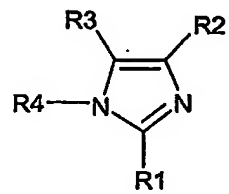
The NCI also provides an *in vivo* screening program to try to identify potential anti-cancer drugs (NCI *In Vivo* Anticancer Drug Screen). Many of the results from this screening program are also available from the NCI/DTP website.

Amongst those compounds tested in one or both of the NCI screens are some aryl imidazole compounds (NCI # 322334, 338970, 144033). None of these three compounds showed any activity in the *In Vivo* Anticancer Drug Screen, even though one of these compounds (NCI # 338970) had been reported as active in Stage0 testing in the Yeast Anticancer Drug Screen. The fact that this compound was active in the yeast screen yet showed no activity in the *in vivo* assay confirms that a positive result in the yeast screen is not necessarily predictive of the utility of a compound as in anti-cancer therapeutic.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a class of compounds which are 2,4,5-trisubstituted imidazole derivatives that have anti-cancer activity. In accordance with an aspect of the present invention there is provided a use of a compound having structural formula (I), or a salt thereof, as an anti-cancer agent:



(I)

wherein:

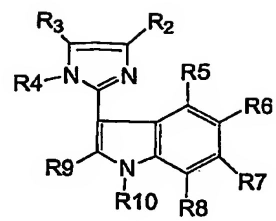
R1 is aryl, substituted aryl, heterocycle, substituted heterocycle, heteroaryl, substituted heteroaryl or amino;

R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl or R2 and R3 when taken together along with the carbon atoms they are attached to, form aryl or substituted aryl, heterocycle, substituted heterocycle, heteroaryl, or substituted heteroaryl and

R4 is hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, substituted alkylthiol, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano, $-S(O)_{0\text{-}2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl.

In accordance with another aspect of the present invention, there is provided a use of a compound having structural formula (I), or a salt thereof, in the preparation of an anti-cancer composition.

In accordance with another aspect of the present invention, there is provided a compound having the structural formula:



II

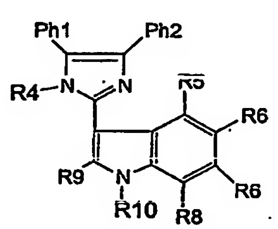
or a salt thereof, wherein:

R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl or R2 and R3 when taken together along with the carbon atoms they are attached form aryl or substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0\text{-}2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

R10 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl, $-CH_2$-aryl, $-CH_2$-heteroaryl.

In accordance with another aspect of the present invention, there is provided a compound having the structural formula:



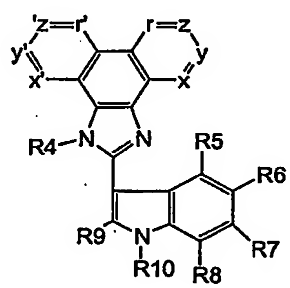
III

or a salt thereof, wherein:

Ph1 and Ph2 are independently selected from phenyl and substituted phenyl;

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0-2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;R10 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl, $-CH_2$-aryl, $-CH_2$-heteroaryl.

In accordance with another aspect of the present invention, there is provided a compound having the structural formula:



VI

or a salt thereof, wherein:

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano or $-S(O)_{0-2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;R10 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl, $-CH_2$-aryl, $-CH_2$-heteroaryl;

x is CR11 or N;

y is CR12 or N;

z is CR13 or N;

r is CR14 or N;

x' is CR15 or N;

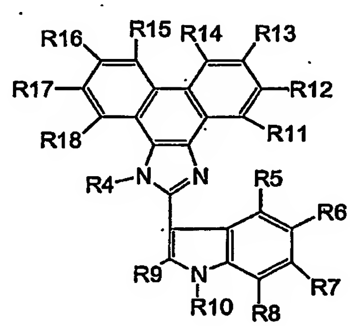
y' is CR16 or N;

z' is CR17 or N;

r' is CR18 or N;

R11, R12, R13, R14, R15, R16, R17 and R18 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In accordance with another aspect of the present invention, there is provided a compound having the structural formula:



VII

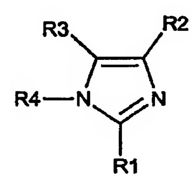
or a salt thereof, wherein:

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0\text{-}2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

R10 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl, $-CH_2$-aryl, $-CH_2$-heteroaryl;

R11, R12, R13, R14, R15, R16, R17 and R18 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, alkenyl, alkenyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In accordance with another embodiment of the present invention, there is provided a use of a therapeutically effective amount of a compound of formula I:



(I)

wherein:

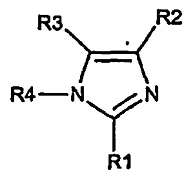
R1 is aryl, substituted aryl, heterocycle, substituted heterocycle, heteroaryl, substituted heteroaryl or amino;

R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl or R2 and R3 when taken together along with the carbon atoms they are attached to, form aryl or substituted aryl, and

R4 is hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0-2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl,

to inhibit neoplastic cell growth or proliferation in a mammal.

In accordance with another embodiment of the present invention, there is provided a use of a therapeutically effective amount of a compound of formula I:



(I)

wherein:

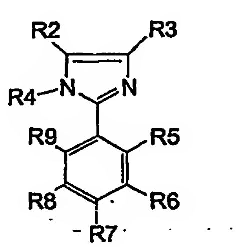
R1 is aryl, substituted aryl, heterocycle, substituted heterocycle, heteroaryl, substituted heteroaryl or amino;

R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl or R2 and R3 when taken together along with the carbon atoms they are attached to, form aryl or substituted aryl, and

R4 is hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0\text{-}2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl,

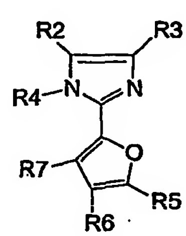
in the treatment of cancer in a mammal in need thereof.

In accordance with another embodiment of the present invention, there is provided a compound selected from the compounds of structural formulae:



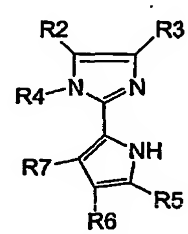
VIII

or



IX

or



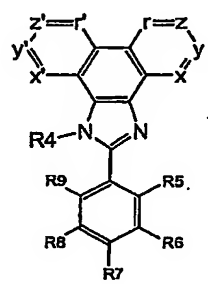
X

wherein:

R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl or R2 and R3 when taken together along with the carbon atoms they are attached to, form a aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

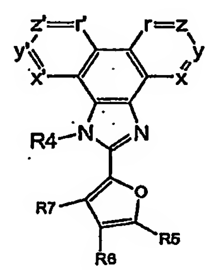
R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0-2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl.

In accordance with another embodiment of the present invention, there is provided a compound selected from the compounds of structural formulae:



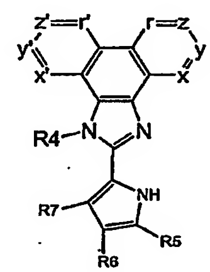
XI

or



XII

or



XIII

or a salt thereof, wherein:

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0-2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

x is CR11 or N;
y is CR12 or N;
z is CR13 or N;
r is CR14 or N;
x' is CR15 or N;
y' is CR16 or N;
z' is CR17 or N;
r' is CR18 or N;
R11, R12, R13, R14, R15, R16, R17 and R18 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In accordance with another embodiment of the present invention, there is provided a use of a compound of formula (I) in the manufacture of a medicament for the inhibition of neoplastic cell growth or proliferation.

In accordance with another embodiment of the present invention, there is provided a use of a compound of formula (I) in the maunfacture of a medicament for the treatment of cancer.

In accordance with another aspect of the present invention, there is provided an anti-cancer composition comprising an effective amount of a compound having structural formula (I), or a salt thereof, and a carrier, diluent or excipient.

In accordance with another aspect of the present invention there is provided a method of inhibiting neoplastic cell growth or proliferation in a mammal comprising administering to said mammal a therapeutically effective amount of a compound selected from the compounds of general formula (I), (II), (III), (IV), (V), (VI), (VII), (VIII), (IX), (X), (XI), (XII) and (XIII), or a salt thereof.

In accordance with another aspect of the present invention there is provided a method of treating cancer in a mammal comprising administering to said mammal a therapeutically effective amount of a compound selected from the compounds of general formula (I), (II), (III), (IV), (V), (VI) , (VII), (VIII), (IX), (X), (XI), (XII) and (XIII), or a salt thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the effects of a compound **92** on the proliferation of various cancer cell lines *in vitro.*

Figure 2 depicts the effects of a compound **28** on the proliferation of various cancer cell lines *in vitro.*

Figure 3 depicts the effects of a compound 50 on the proliferation of various cancer cell lines *in vitro.*

Figure 4 depicts the effects of a compound **42** on the proliferation of various cancer cell lines *in vitro.*

Figure 5A-C depicts the effects of various concentrations of a compound **45** on the proliferation of cancer cell lines *in vitro* at different time intervals.

Figure 6A-C depicts the effects of various concentrations of a compound **45** on the proliferation of cancer cell lines *in vitro* at different time intervals.

Figure 7 depicts the effects of compounds **83** and **99** on the proliferation of LS 513 colon carcinoma cells *in vitro.*

Figure 8 depicts the effects of compounds of Formula I on the proliferation of HT-29 colon adenocarcinoma cells *in vitro.*

Figure 9A-C present the cancer cell lines used to in the NCI screen used to determine the ability of compounds of Formula I to inhibit cancer cell proliferation *in vitro*.

Figure 10A depicts the average and mean GI_{50} values for various compounds of Formula I for a number of cancer cell lines; **B** depicts the average GI_{50} values for compound **45** by cancer cell type and **C** depicts the average total growth inhibition (TGI) for compound **45** by cancer cell type.

Figure 11 depicts the inhibition of H460 NSCLC cell proliferation *in vitro* by compounds of Formula I.

Figure 12 depicts the inhibition of HT-29 colon carcinoma cell proliferation *in vitro* by compounds of Formula I.

Figure 13 depicts the inhibition of HT-29 colon carcinoma cell proliferation *in vitro* by compounds of Formula I.

Figure 14 depicts the effects of compounds of Formula I on the growth of HT-29 colon adenocarcinoma cells *in vivo* in CD-1 nude mice.

Figure 15 depicts the effects of compounds of Formula I on the average weight of tumours in CD-1 nude mice (average weight per group of mice).

Figure 16 depicts the effects of compounds of Formula I on the weight of tumours in CD-1 nude mice (individual tumour weights).

Figure 17 depicts the effects of compounds of Formula I on the growth of HT-29 colon adenocarcinoma cells *in vivo* in CD-1 nude mice.

Figure 18 depicts the effect of compound **45** on the growth of HepG2 hepatocarcinoma cells *in vivo* in CD-1 nude mice in terms of **A** tumour size, and **B** tumour weight.

Figure 19 depicts the effects of compounds **45, 33** and **90** on the activity of various human kinases.

Figure 20 depicts the subcellular location of compound **45** in HT-29 colon adenocarcinoma cells **(A, B)**; of doxorubicin in HT-29 colon adenocarcinoma cells

(C); of compound **45** in A498 renal cancer cells **(D)**, and of compound **45** in C8161 melanoma cells **(E)**.

Figure 21 depicts the formation of vacuoles in HT-29 colon adenocarcinoma cells treated with compound **45** or doxorubicin.

Figure 22 depicts the effects of compound **45** on the cell cycle in HT-29 colon adenocarcinoma cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a class of 2,4,5-trisubstituted imidazole compounds and for their use as anti-cancer agents. The present invention further provides for methods of inhibiting neoplastic cell growth and/or proliferation in an animal by administering to the animal an effective amount of a compound of Formula I, either alone or in combination with one or more standard chemotherapeutics.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

The terms are defined as follows:

The term "halogen" refers to fluorine, bromine, chlorine, and iodine atoms.

The term "hydroxyl" refers to the group -OH.

The term "thiol" or "mercapto" refers to the group -SH, and $-S(O)_{0-2}$.

The term "lower alkyl" refers to a straight chain or branched alkyl group of one to ten carbon atoms or a cyclic alkyl group of three to ten carbon atoms. This term is further exemplified by such groups as methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, t-butyl, 1-butyl (or 2-methylpropyl), cyclopropylmethyl, *i*-amyl, *n*-amyl, hexyl and the like.

The term "substituted lower alkyl" refers to lower alkyl as just described including one or more groups such as hydroxyl, thiol, alkylthiol, halogen, alkoxy, amino, amido, carboxyl, cycloalkyl, substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, carboxyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, alkyl cycloheteroalkyl, nitro, cyano. These groups may be attached to any carbon atom of the lower alkyl moiety.

The term "lower alkenyl" refers to a straight chain or branched hydrocarbon of two to ten carbon atoms or a cyclic hydrocarbon of three to ten carbon atoms, having at least one carbon to carbon double bond.

The term "substituted lower alkenyl" refers to lower alkenyl as just described including one or more groups such as hydroxyl, thiol, alkylthiol, halogen, alkoxy, amino, amido, carboxyl, cycloalkyl, substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, carboxyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, alkyl, alkenyl, alkynyl, alkyl alkenyl, alkyl alkynyl; alkyl cycloalkyl, alkyl cycloheteroalkyl, nitro, cyano. These groups may be attached to any carbon atom to produce a stable compound.

The term "lower alkynyl" refers to a straight chain or branched hydrocarbon of two to ten carbon atoms having at least one carbon to carbon triple bond.

The term "substituted lower alkynyl" refers to lower alkynyl as just described including one or more groups such as hydroxyl, thiol, alkylthiol, halogen, alkoxy, amino, amido, carboxyl, cycloalkyl, substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, carboxyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl; alkyl, alkenyl, alkynyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, alkyl cycloheteroalkyl, nitro, cyano. These groups may be attached to any carbon atom to produce a stable compound.

The term "alkoxy" refers to the group -OR, where R is lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroalkyl,

heteroarylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, or substituted cycloheteroalkyl as defined below.

The term "alkylthio" denotes the group -SR, $-S(O)_{n=1-2}$ -R, where R is lower alkyl, substituted lower alkyl, aryl, substituted aryl, aralkyl or substituted aralkyl as defined below.

The term "acyl" refers to groups -C(O)R, where R is hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, cycloalkyl or substituted cycloalkyl.

The term "aryloxy" refers to groups -OAr, where Ar is an aryl, substituted aryl, heteroaryl, or substituted heteroaryl group as defined below.

The term "amino" refers to the group NRR', where R and R' may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl, cycloalkyl, or substituted heteroaryl as defined below, acyl, D or L aminoacid or a protected form thereof.

The term "amido" refers to the group -C(O)NRR', where R and R' may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, substituted hetaryl as defined below.

The term "carboxyl" refers to the group -C(O)OR, where R may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, substituted hetaryl and the like as defined.

The terms "aryl" or "Ar" refer to an aromatic carbocyclic group having at least one aromatic ring (e.g., phenyl or biphenyl) or multiple condensed rings in which at least one ring is aromatic, (e.g., 1,2,3,4-tetrahydronaphthyl, naphthyl, anthryl, phenanthryl, 9-fluorenyl, dibenzocycloheptatrienyl etc.).

The term "substituted aryl" refers to aryl optionally substituted with one or more functional groups, e.g., halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, trifluoromethyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, substituted heterocycle,

heteroaryl, substituted heteroaryl, heteroalkyl, substituted heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, sulfamido, cyano or –N=CRR', wherein R and R' are independemtly selected from H, alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, substituted heterocycle, heteroaryl or substituted heteroaryl.

The term "heterocycle" refers to a saturated, unsaturated, or aromatic carbocyclic group having a single ring (e.g., morpholino, pyridyl or furyl) or multiple condensed rings (e.g., naphthpyridyl, quinoxalyl, quinolinyl, indolizinyl, indanyl or benzo[b]thienyl) and having at least one hetero atom, such as N, O or S, within the ring.

The term "substituted heterocycle" refers to heterocycle optionally substituted with, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, trifluoromethyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, substituted heterocycle, heteroaryl, substituted heteroaryl, heteroalkyl, substituted heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, sulfamido or cyano and the like.

The terms "heteroaryl" or "hetaryl" refer to a heterocycle in which at least one heterocyclic ring is aromatic.

The term "substituted heteroaryl" refers to a heterocycle optionally mono or poly substituted with one or more functional groups, e.g., halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, trifluoromethyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, substituted heterocycle, heteroaryl, substituted heteroaryl, heteroalkyl, substituted heteroalkyl, cycloalkyl, ~~substituted cycloalkyl,~~ alkylcycloalkyl, alkylcycloheteroalkyl, nitro, sulfamido, cyano or –N=CRR', wherein R and R' are independemtly selected from H, alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, substituted heterocycle, heteroaryl or substituted heteroaryl and the like.

The term "aralkyl" refers to the group -R-Ar where Ar is an aryl group and R is lower alkyl or substituted lower alkyl group. Aryl groups can optionally be unsubstituted or substituted with, e.g., halogen, lower alkyl, alkoxy, alkyl thio, trifluoromethyl, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

The term "heteroalkyl" refers to the group -R-Het where Het is a heterocycle group and R is a lower alkyl group. Heteroalkyl groups can optionally be unsubstituted or substituted with e.g., halogen, lower alkyl, lower alkoxy, lower alkylthio, trifluoromethyl, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

The term "heteroarylalkyl" refers to the group -R-HetAr where HetAr is an heteroaryl group and R lower alkyl or substituted loweralkyl. Heteroarylalkyl groups can optionally be unsubstituted or substituted with, e.g., halogen, lower alkyl, substituted lower alkyl, alkoxy, alkylthio, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

The term "cycloalkyl" refers to a cyclic or polycyclic alkyl group containing 3 to 15 carbon. For polycyclic groups, these may be multiple condensed rings in which one of the distal rings may be aromatic (e.g. tetrahydronaphthalene, etc.).

The term "substituted cycloalkyl" refers to a cycloalkyl group comprising one or more substituents with, e.g halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, trifluoromethyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, sulfamido or cyano and the like.

The term "cycloheteroalkyl" refers to a cycloalkyl group wherein one or more of the ring carbon atoms is replaced with a heteroatom (e.g., N, O, S or P).

The term "substituted cycloheteroalkyl" refers to a cycloheteroalkyl group as herein defined which contains one or more substituents, such as halogen, lower alkyl, lower

alkoxy, lower alkylthio, trifluoromethyl, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

The term "alkyl cycloalkyl" refers to the group -R-cycloalkyl where cycloalkyl is a cycloalkyl group and R is a lower alkyl or substituted lower alkyl. Cycloalkyl groups can optionally be unsubstituted or substituted with e.g. halogen, lower alkyl, lower alkoxy, lower alkylthio, trifluoromethyl, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, alkylthio, thiol, sulfamido and the like.

The terms "therapy" and "treatment," as used interchangeably herein, refer to an intervention performed with the intention of alleviating the symptoms associated with, preventing the development of, or altering the pathology of a disease, disorder or condition. Thus, the terms therapy and treatment are used in the broadest sense, and include the prevention (prophylaxis), moderation, reduction, and curing of a disease, disorder or condition at various stages. Those in need of therapy/treatment include those already having the disease, disorder or condition as well as those prone to, or at risk of developing, the disease, disorder or condition and those in whom the disease, disorder or condition is to be prevented.

The term "subject" or "patient," as used herein, refers to an animal in need of treatment.

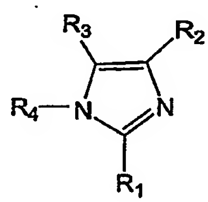
The term "animal," as used herein, refers to both human and non-human animals, including, but not limited to, mammals, birds and fish.

Administration of the compounds of the invention "in combination with" one or more further therapeutic agents, is intended to include simultaneous (concurrent) administration and consecutive administration. Consecutive administration is intended to encompass various orders of administration of the therapeutic agent(s) and the compound(s) of the invention to the subject.

As used herein, the term "about" refers to a +/-10% variation from the nominal value. It is to be understood that such a variation is always included in any given value provided herein, whether or not it is specifically referred to.

I. 2,4,5-Trisubstituted Imidazole Compounds

The present invention provides compounds of the general formula (I):



(I)

or a salt thereof, wherein:

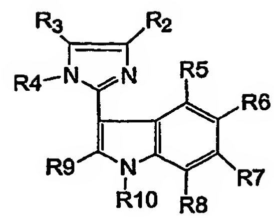
R1 is aryl, substituted aryl, heterocycle, substituted heterocycle, heteroaryl, substituted heteroaryl or amino;

R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl or R2 and R3 when taken together along with the carbon atoms they are attached to, form aryl or substituted aryl, heterocycle, substituted heterocycle, heteroaryl, or substituted heteroaryl;

R4 is hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0-2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl.

In another embodiment of the present invention, the compound of formula (I) is other than Nortopsentin A, Nortopsentin B, Nortopsentin C and Nortopsentin D.

In another embodiment of the present invention, the compound of Formula I includes the compound of the structural formula:



II

or a salt thereof, wherein:

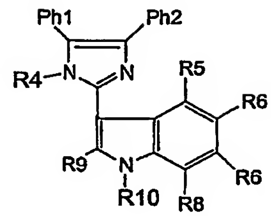
R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl or R2 and R3 when taken together along with the carbon atoms they are attached form aryl or substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0-2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

R10 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl, $-CH_2$-aryl, $-CH_2$-heteroaryl.

In another embodiment of the invention, the compound of Formula II is other than Nortopsentin A, Nortopsentin B, Nortopsentin C and Nortopsentin D.

In another embodiment of the present invention, the compound of Formula II includes the compound of the structural formula III:



III

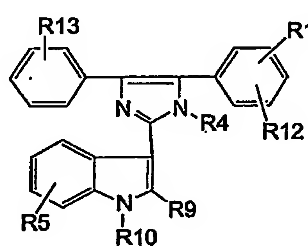
or a salt thereof, wherein:

Ph1 and Ph2 are independently selected from phenyl and substituted phenyl;

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0\text{-}2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

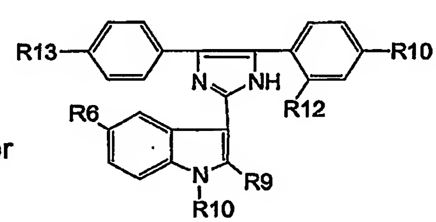
R10 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alknyyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl.

In another embodiment of the invention, the compound of Formula III is selected from:



IV

or



V

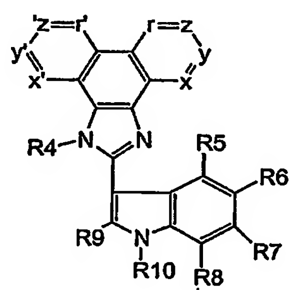
or a salt thereof, wherein:

R5, R6, R9, R11, R12 and R13 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower

alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano;

R10 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl.

In another embodiment of the present invention, the compound of Formula I includes the compound of the structural formula:



VI

or a salt thereof, wherein:

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0\text{-}2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

x is CR11 or N;

y is CR12 or N;

z is CR13 or N;

r is CR14 or N;

x' is CR15 or N;

y' is CR16 or N;

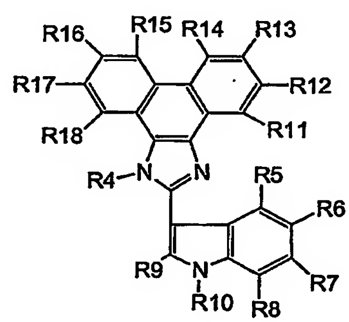
z' is CR17 or N;

r' is CR18 or N;

R10 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl.

R11, R12, R13, R14, R15, R16, R17 and R18 are independently selected from hydrogen,. halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In another embodiment of the present invention, the compound of Formula I includes the compound of the structural formula:



VII

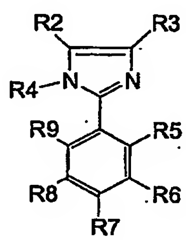
or a salt thereof, wherein:

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano or $-S(O)_{0\text{-}2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

R10 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl;

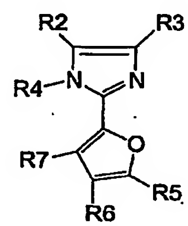
R11, R12, R13, R14, R15, R16, R17 and R18 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In another embodiment of the present invention, the compound of Formula I includes the compound of the strutural formula:



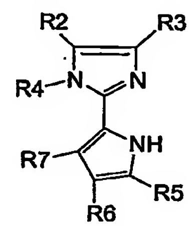
VIII

or



IX

or



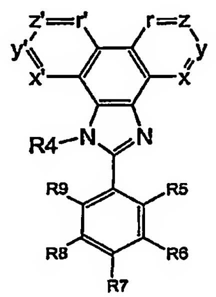
X

wherein:

R2 and R3 are independently aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl or R2 and R3 when taken together along with the carbon atoms they are attached to, form a aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

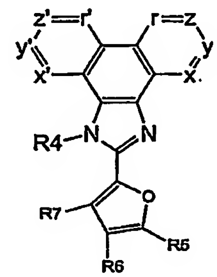
R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, ~~carboxyl~~, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0\text{-}2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl.

In another embodiment of the present invention, the compound of Formula I includes the compound of the structural formula:



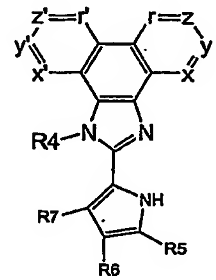
XI

or



XII

or



XIII

or a salt thereof, wherein:

R4, R5, R6, R7, R8 and R9 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0-2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl;

x is CR11 or N;

y is CR12 or N;

z is CR13 or N;

r is CR14 or N;

x' is CR15 or N;

y' is CR16 or N;

z' is CR17 or N;

r' is CR18 or N;

R11, R12, R13, R14, R15, R16, R17 and R18 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl,

aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

In another embodiment, in the compounds of formula (XI) at least one of R11 to R18 is other than H.

In another embodiment, in the compounds of formula (XI) at least one of x, y, z, r, x', y', z' or r' is nitrogen.

In another embodiment the compound of formula (XI) is other than:

2-phenyl-1H-phenanthro[9,10-d]imidazole;

2-(2-methylphenyl)-1H-phenanthro[9,10-d]imidazole;

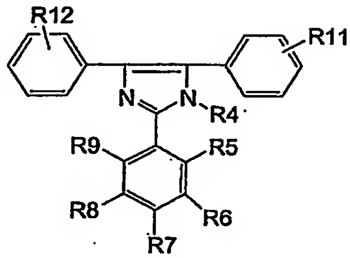
2-(3-iodophenyl)-1H-phenanthro[9,10-d]imidazole;

2-(4-dimethylaminophenyl)-1H-phenanthro[9,10-d]imidazole;

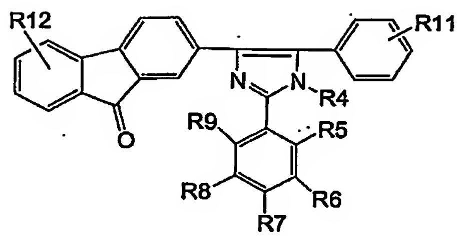
2-(4-nitrophenyl)-1H-phenanthro[9,10-d]imidazole;

1,2-diphenyl-1H-phenanthro[9,10-d]imidazole.

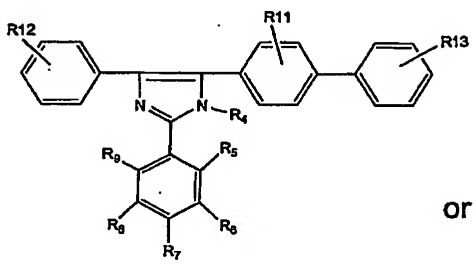
In another embodiment of the invention, the compound of Formula I is selected from:



XIV

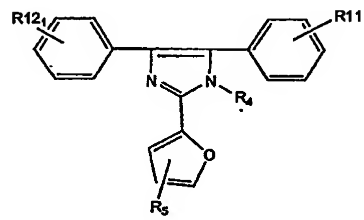


XV



XVI

or

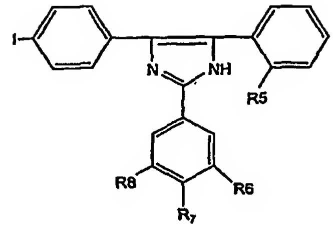


XVII

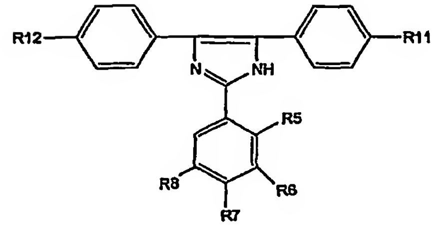
wherein:

R4, R5, R6, R7, R8, R9, R11, R12 and R13 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, cyano or $-S(O)_{0-2}R$ wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl.

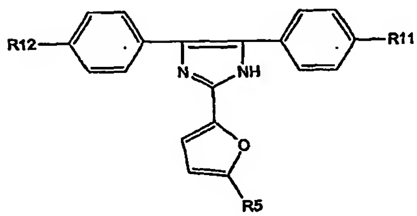
In another embodiment of the invention the compound of Formula I is selected from:



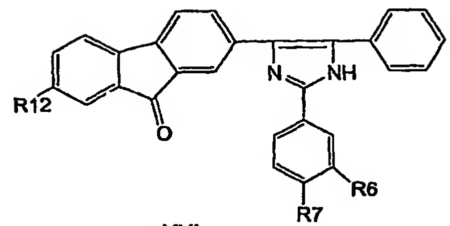
XVIII



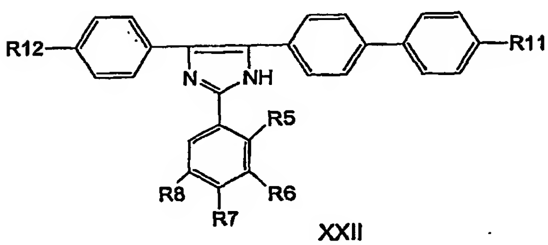
XIX



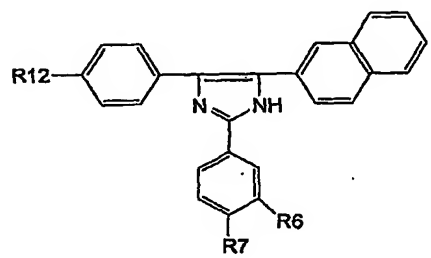
XX



XXI



XXII

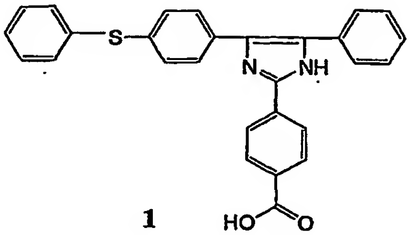


XXIII

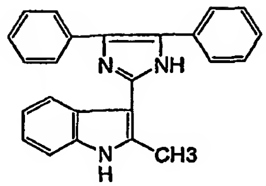
wherein:

R5, R6, R7, R8, R9, R11 and R12 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkylalkenyl, alkyl alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, heteroalkyl, cycloalkyl, substituted cycloalkyl, alkylcycloalkyl, alkylcycloheteroalkyl, nitro, or cyano.

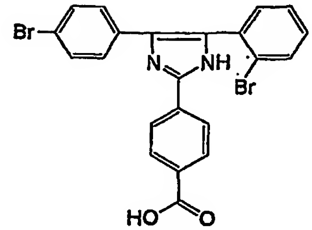
Compounds of the present invention include, but are not limited to the following exemplary compounds:


S
N
NH
HO
O

1


N
NH
N
H
CH3

2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NH
Br
HO
O

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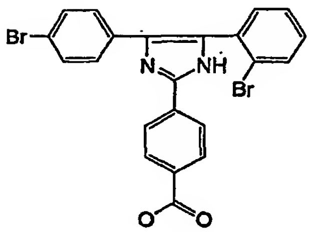
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NH
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O
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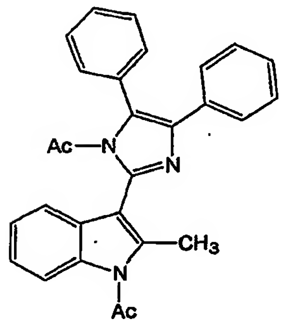
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N
N
CH3
N
Ac

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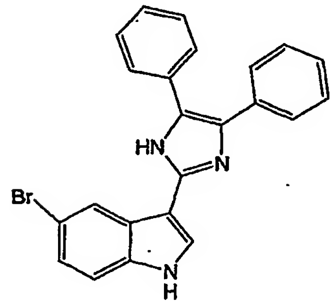
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N
Br
N
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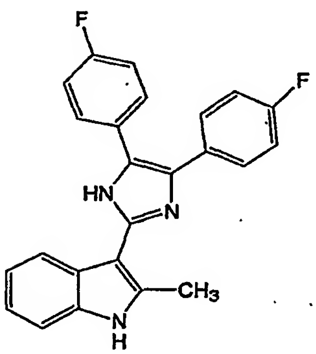
F
F
HN
N
CH3
N
H

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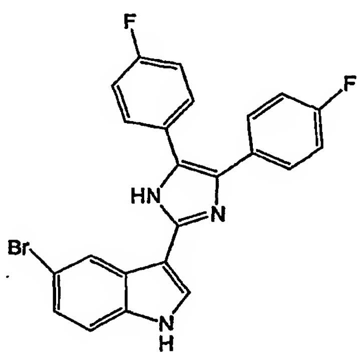
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F
HN
N
Br
N
H

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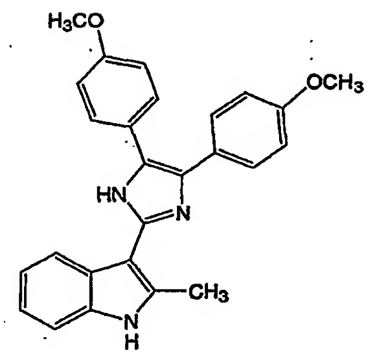
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HN
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N
H

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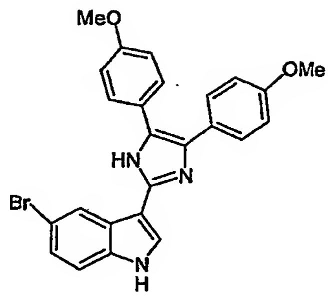

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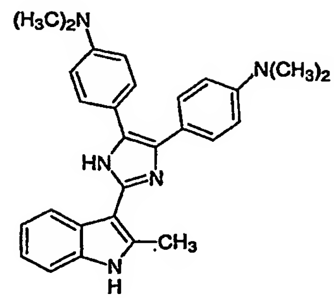

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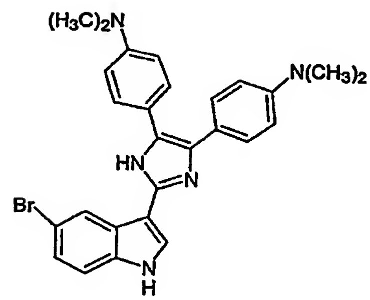

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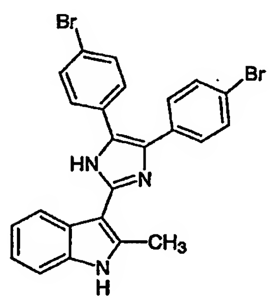

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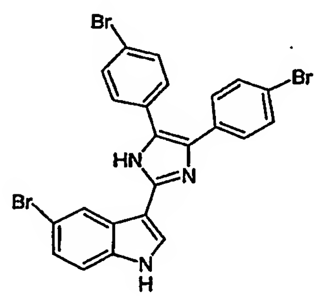

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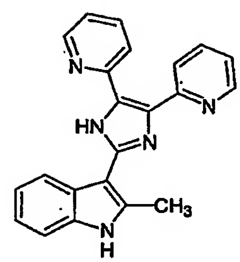

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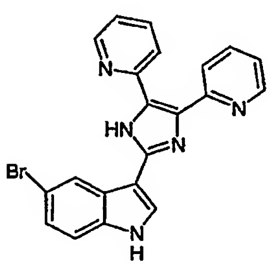

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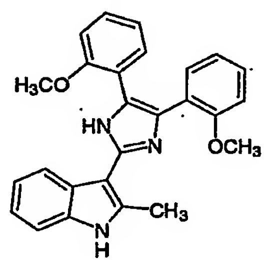

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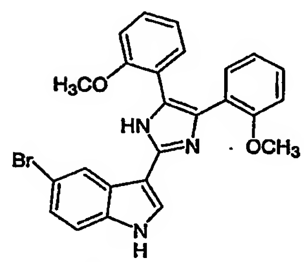

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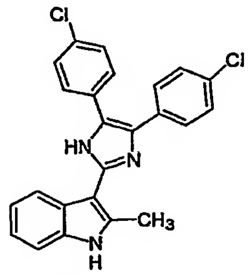

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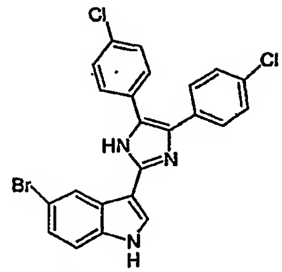

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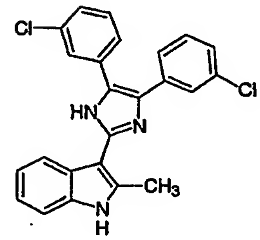

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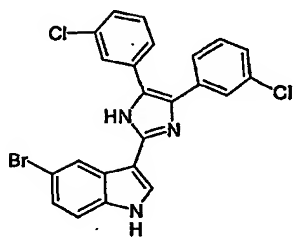

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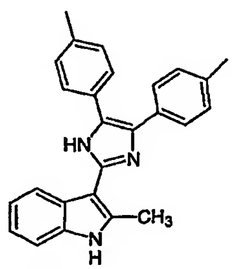

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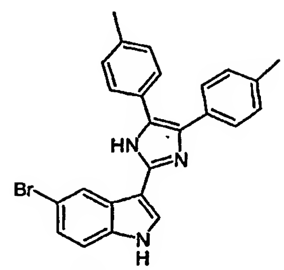

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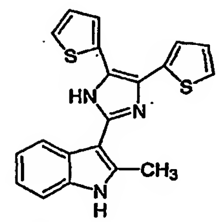

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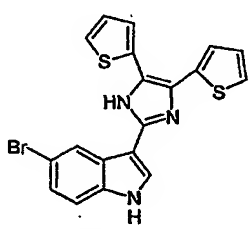

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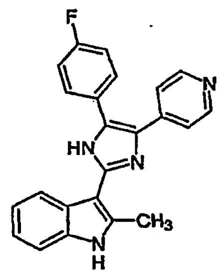

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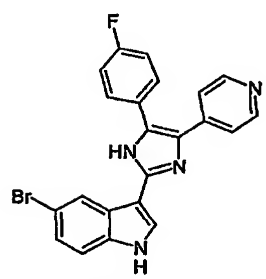

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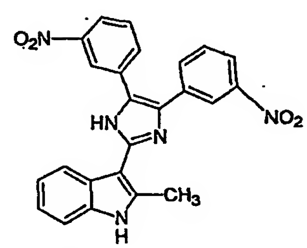

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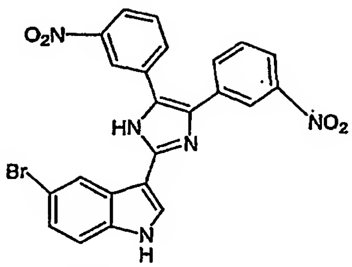

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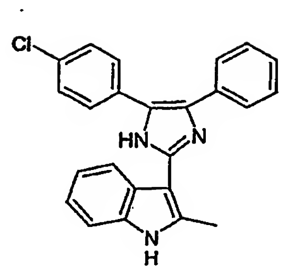

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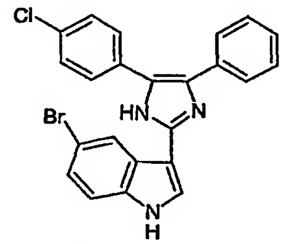

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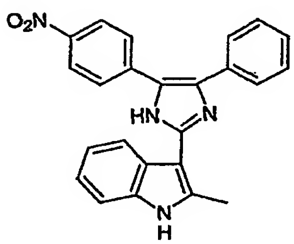

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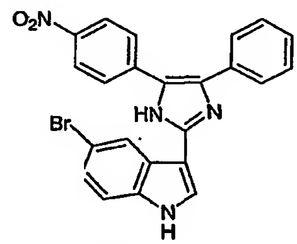

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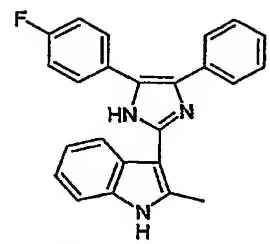

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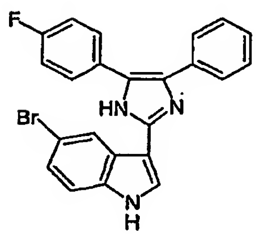

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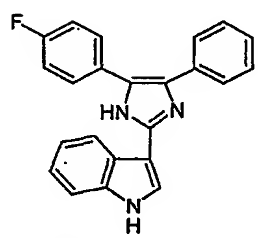

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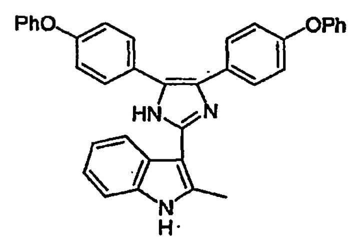

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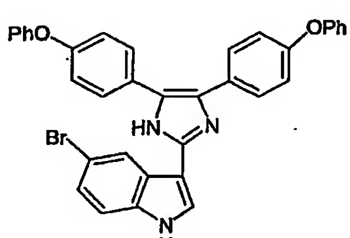

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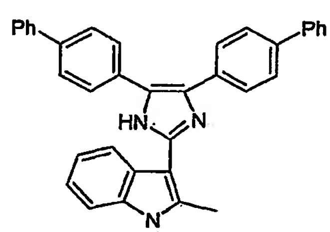

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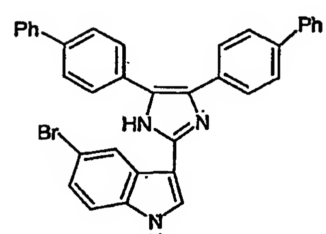

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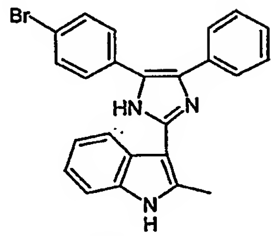
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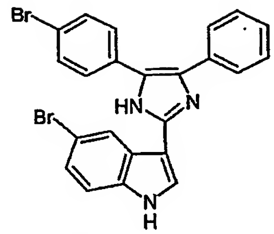
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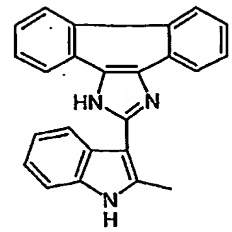
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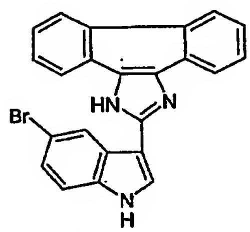
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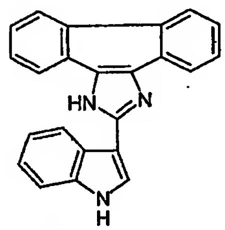
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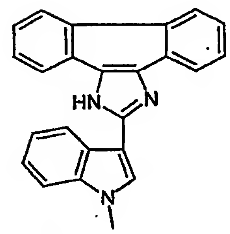
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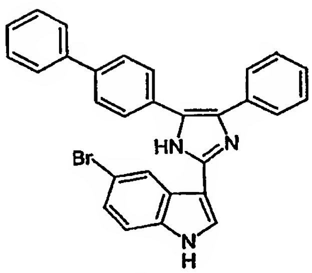
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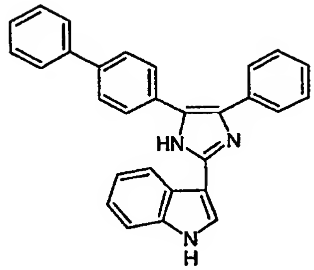
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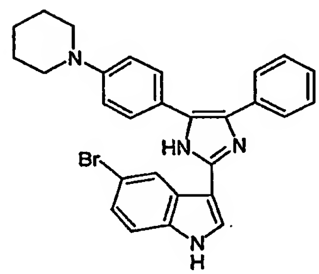
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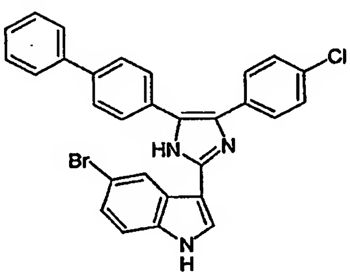
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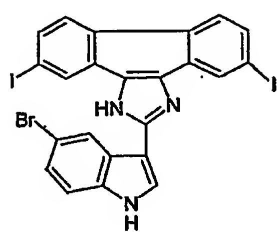
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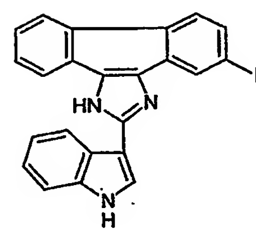
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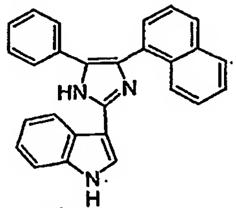

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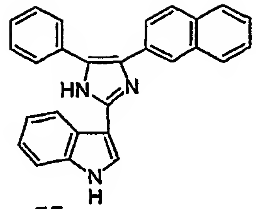

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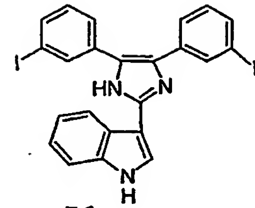

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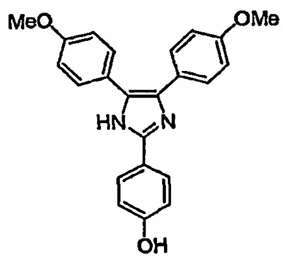

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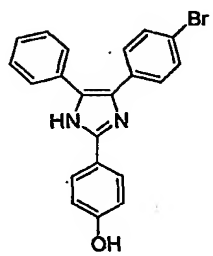

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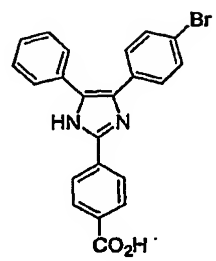

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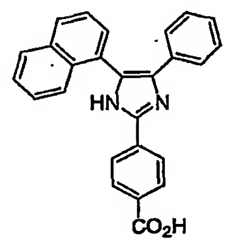

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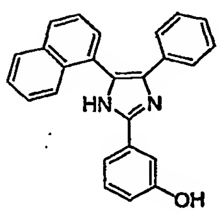

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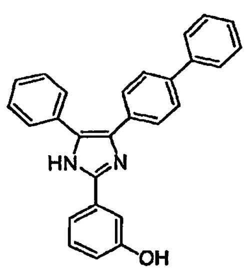

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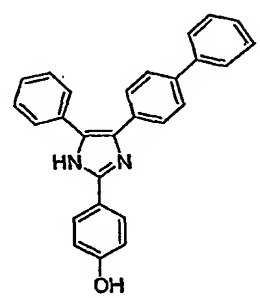

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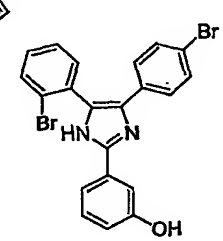

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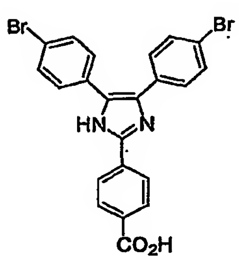

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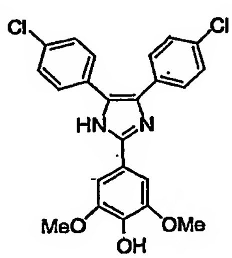

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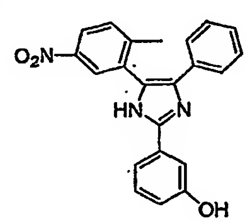

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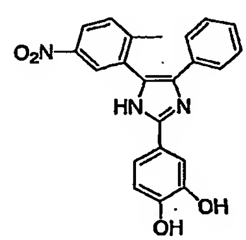

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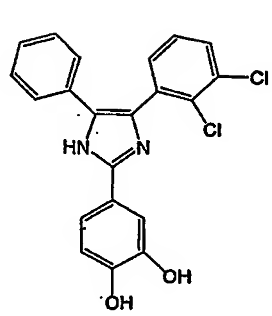

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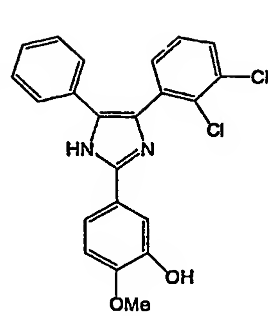

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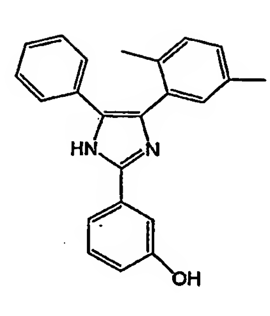

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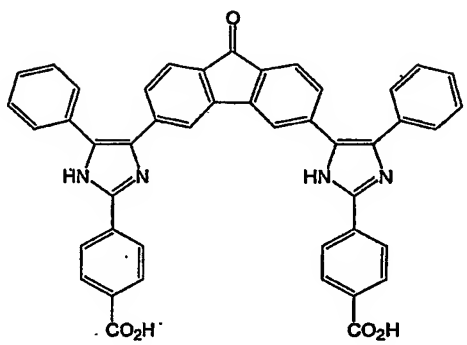

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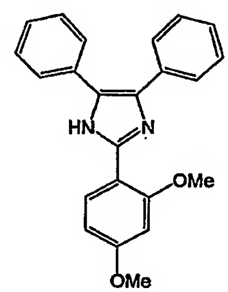

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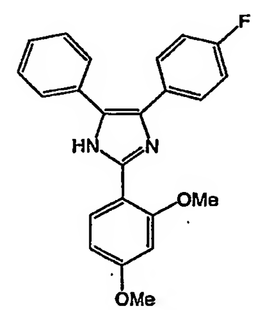

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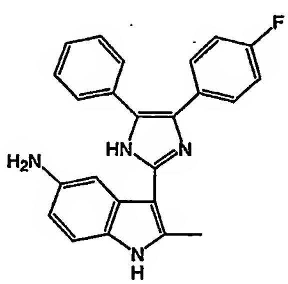

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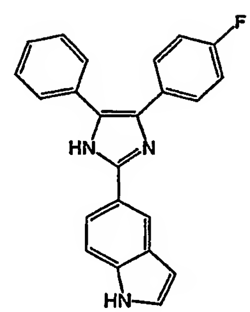

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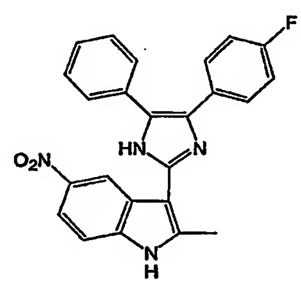

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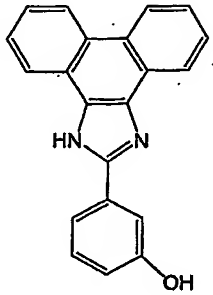

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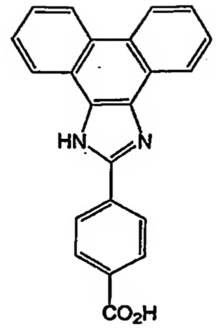

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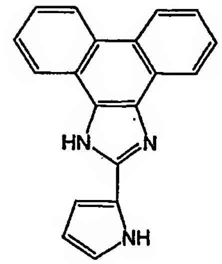

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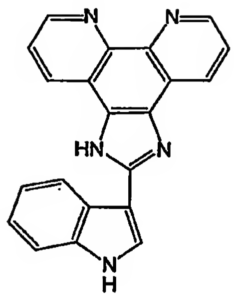

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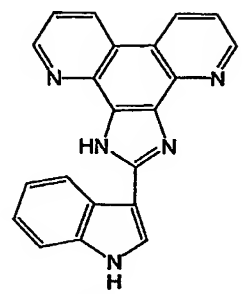

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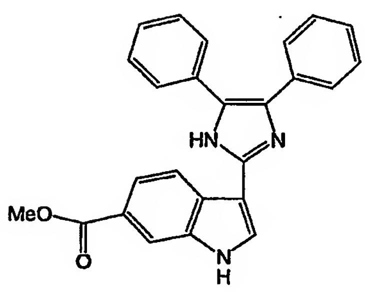

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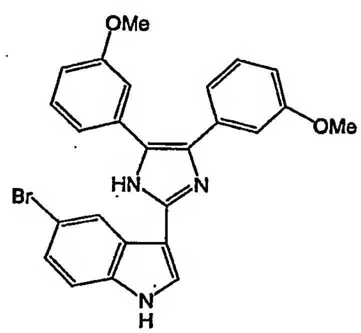

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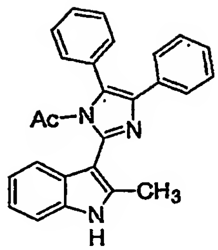

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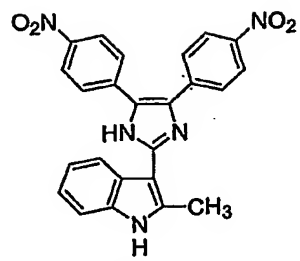

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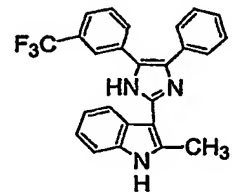

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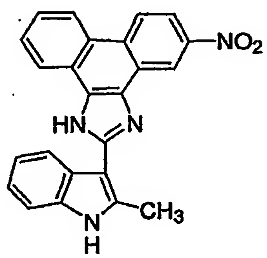

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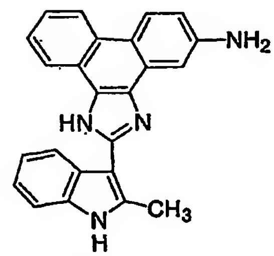

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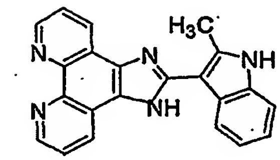

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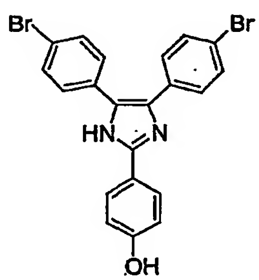

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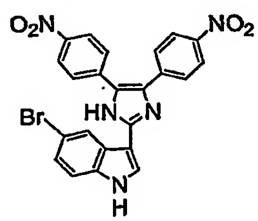

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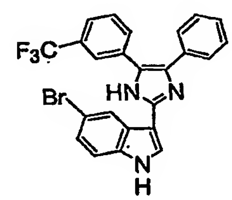

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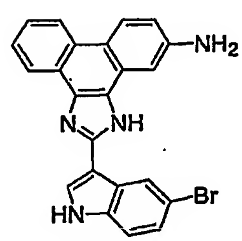

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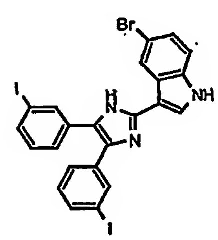

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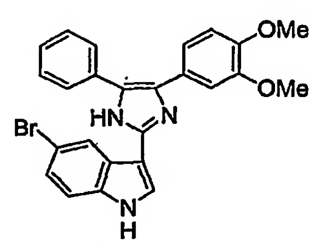

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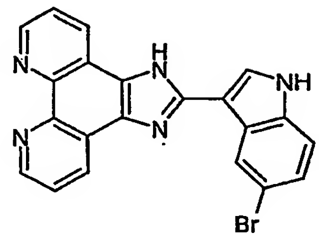

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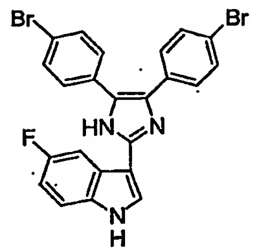

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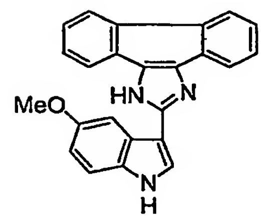

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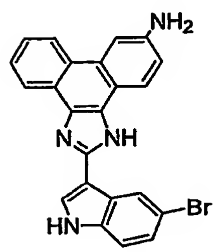

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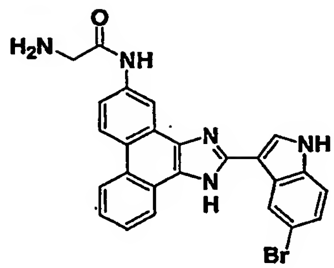

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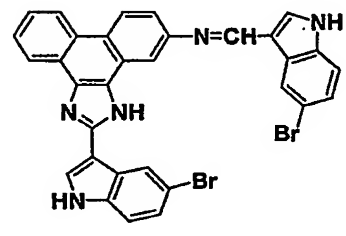

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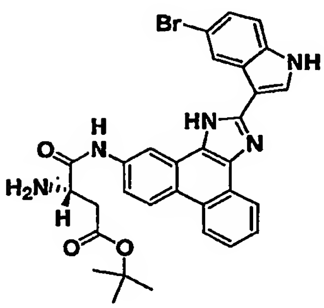

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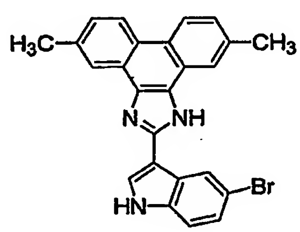

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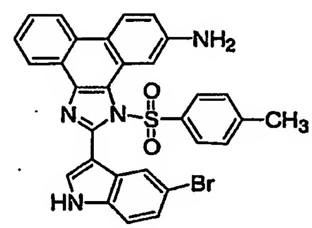

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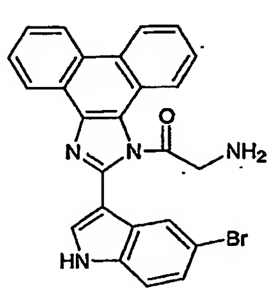

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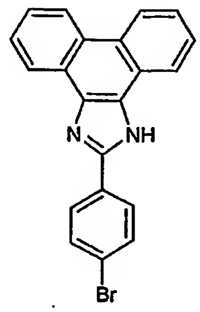

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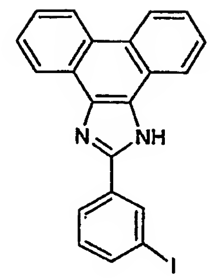

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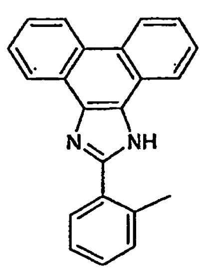

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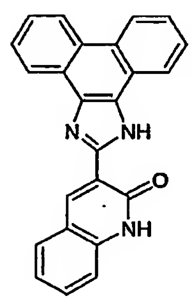

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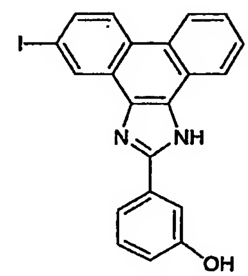

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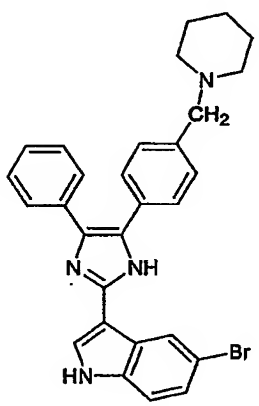

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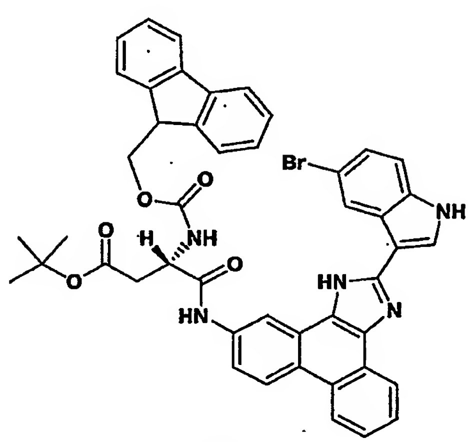

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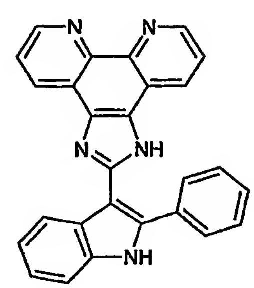

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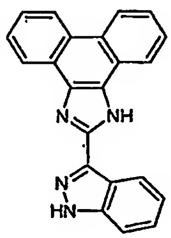

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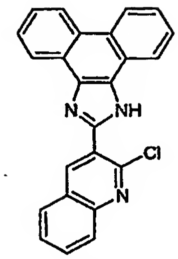

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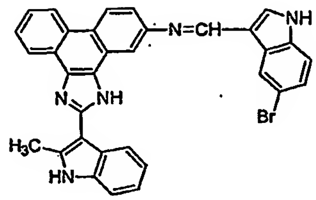

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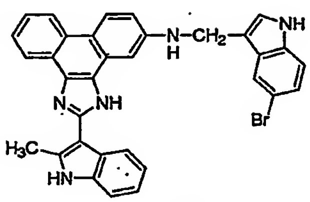

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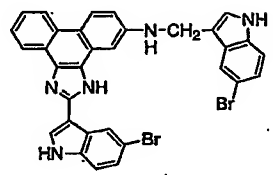

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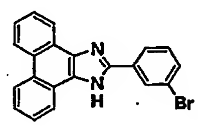

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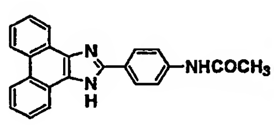

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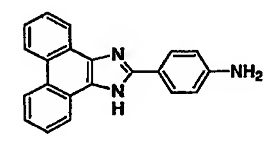

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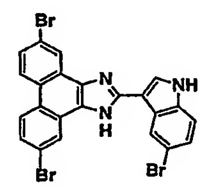

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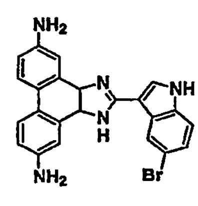

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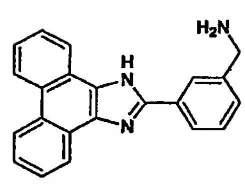

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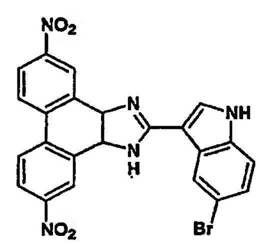

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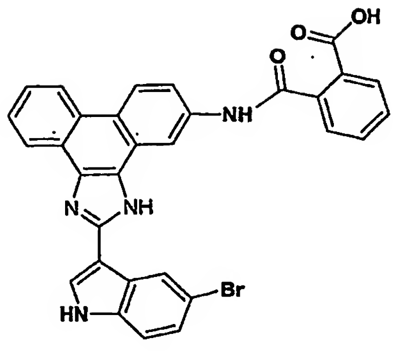

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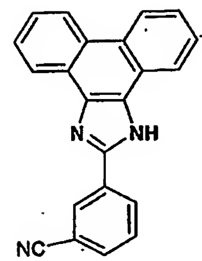

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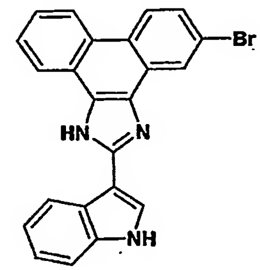

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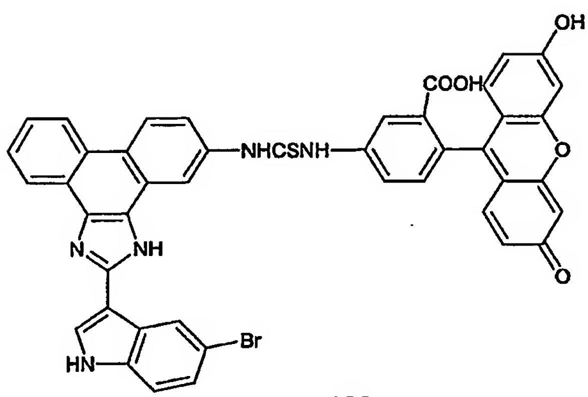

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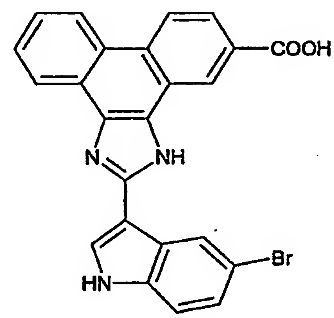

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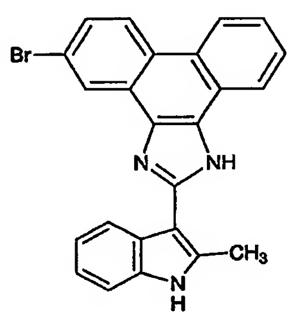

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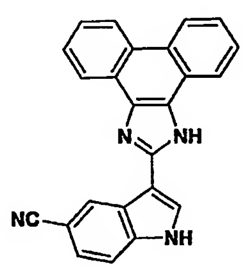

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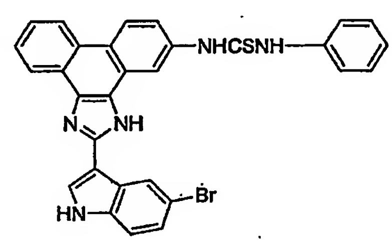

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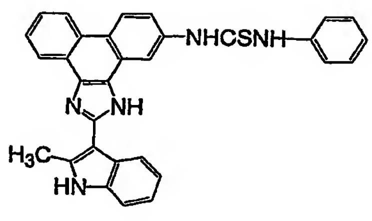

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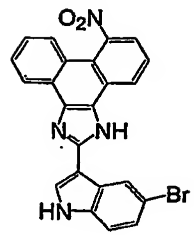

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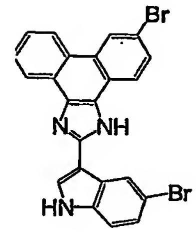

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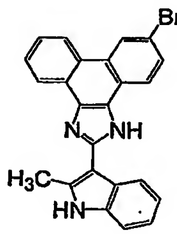

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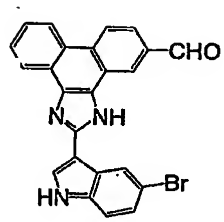

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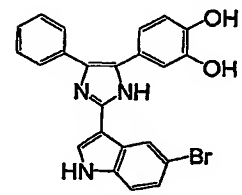

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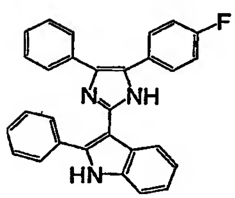

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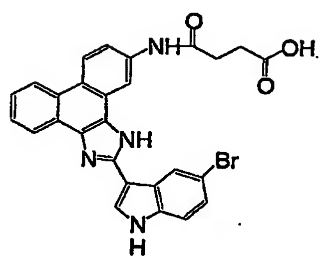

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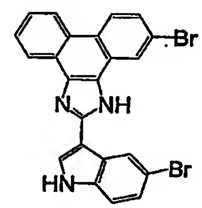

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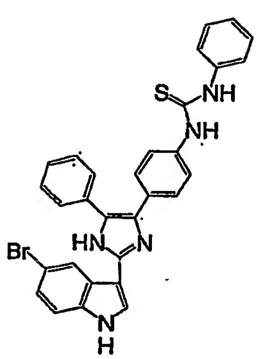

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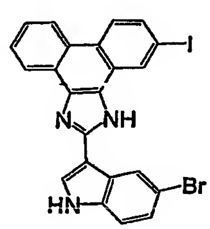

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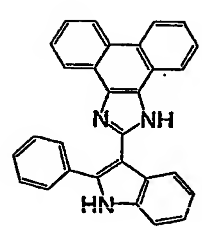

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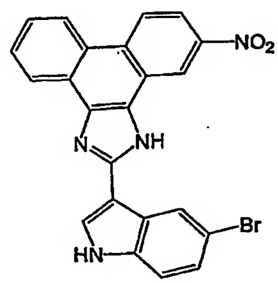

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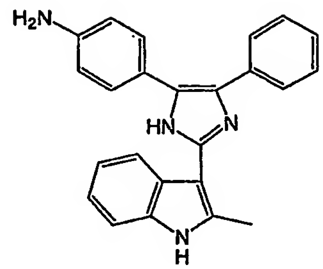

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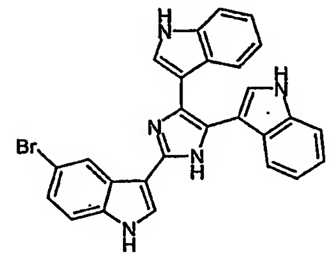

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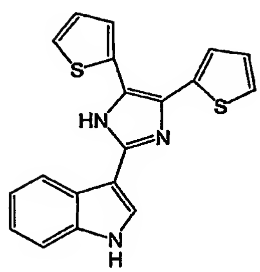

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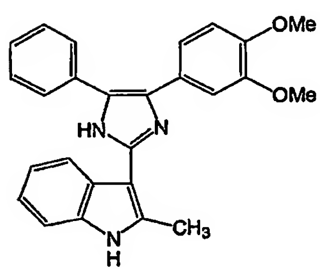

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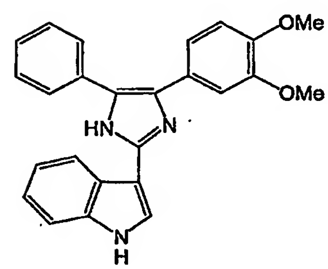

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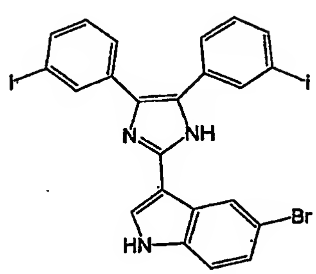

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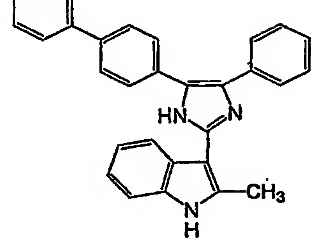

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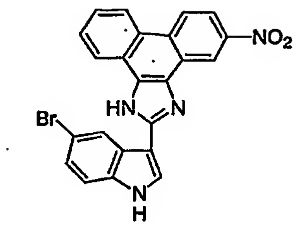

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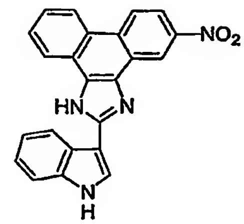

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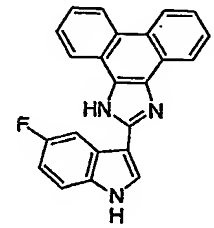

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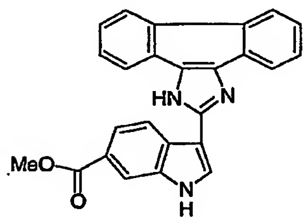

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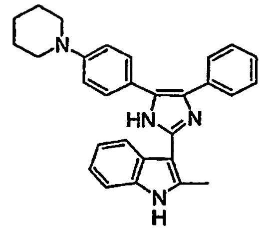

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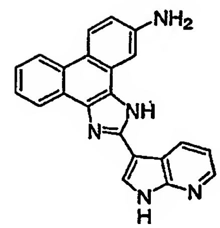

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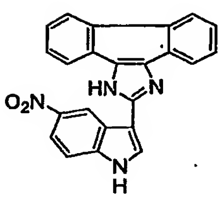

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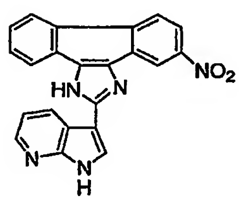

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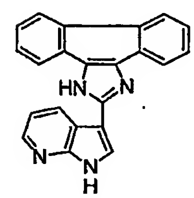

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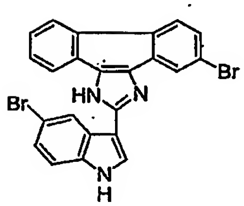

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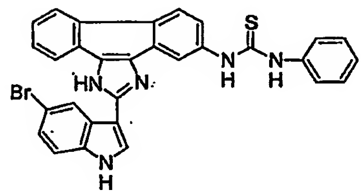

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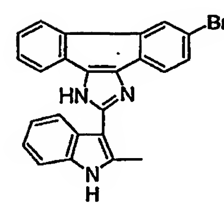

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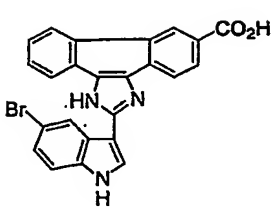

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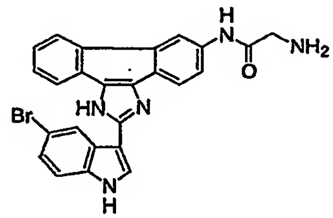

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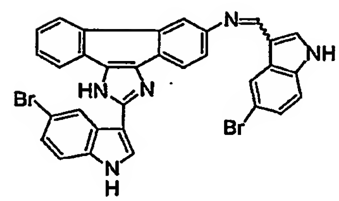

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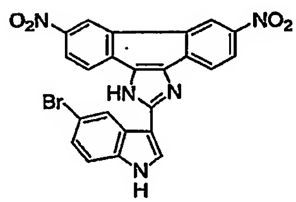

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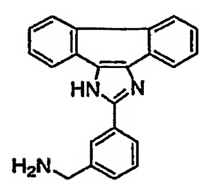

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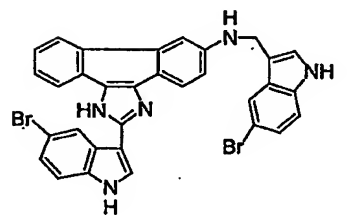

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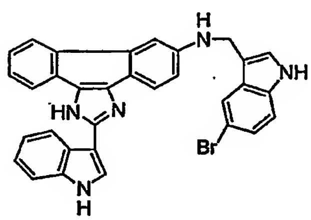

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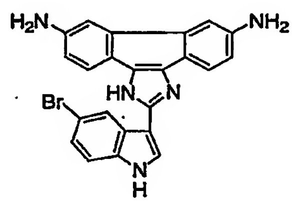

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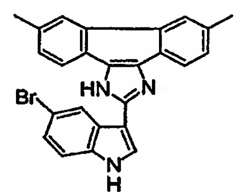

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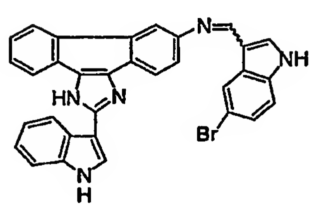

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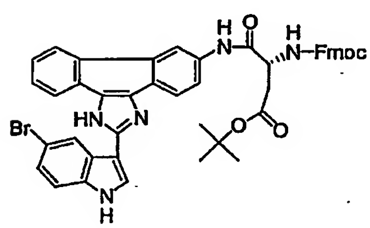

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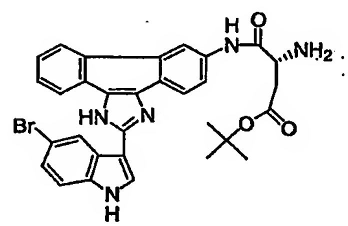

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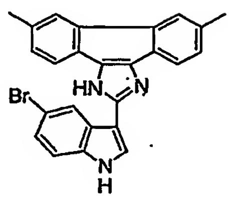

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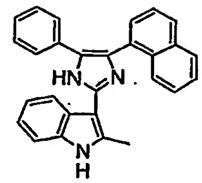

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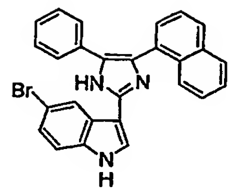

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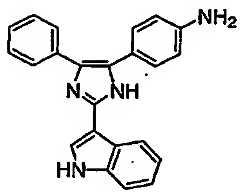

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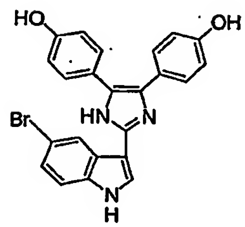

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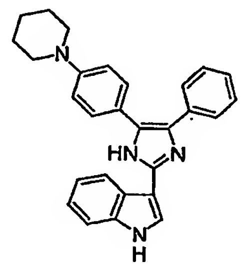

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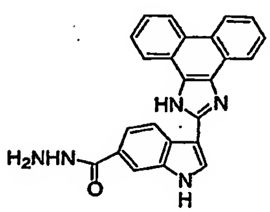

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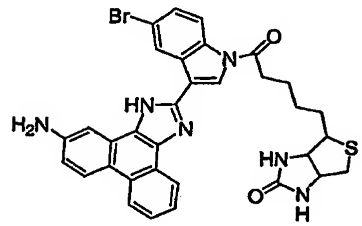

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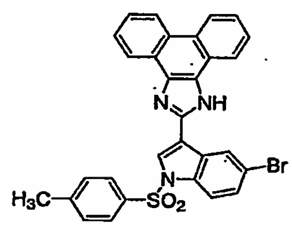

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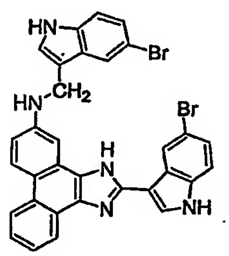

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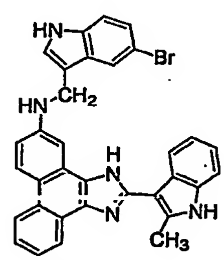

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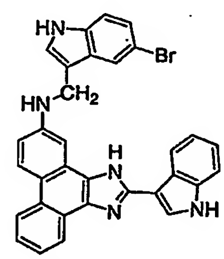

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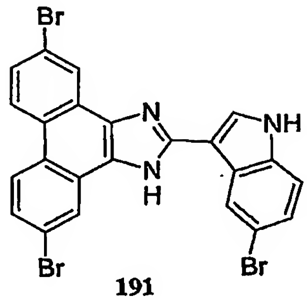

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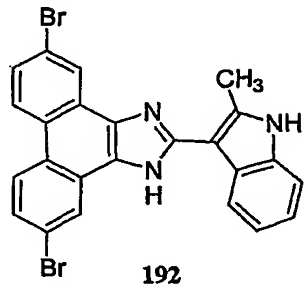

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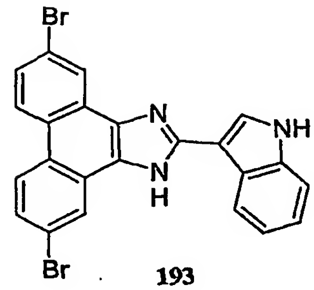

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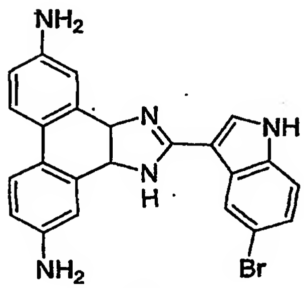

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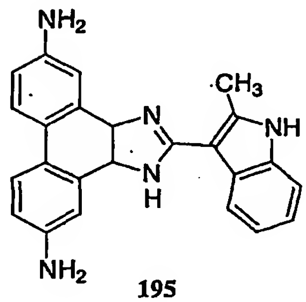

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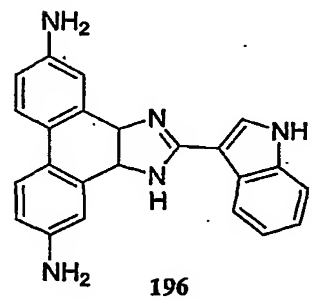

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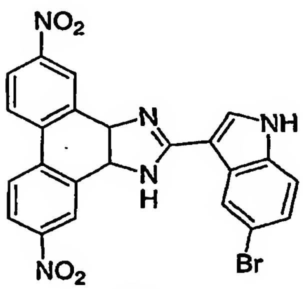

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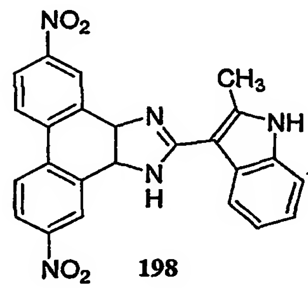

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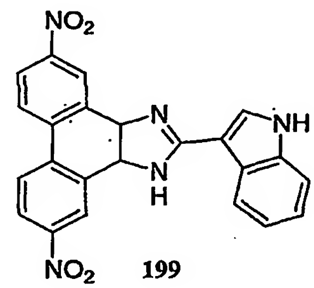

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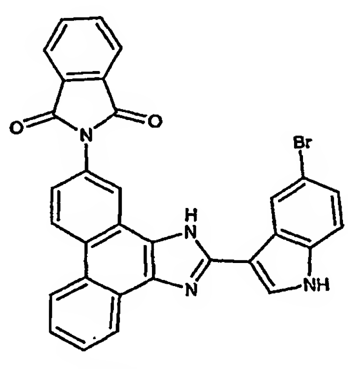

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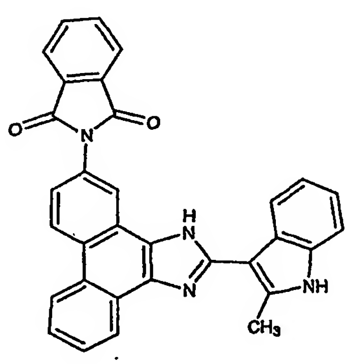

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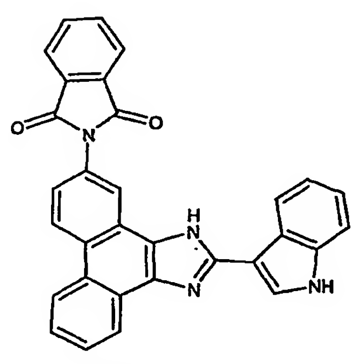

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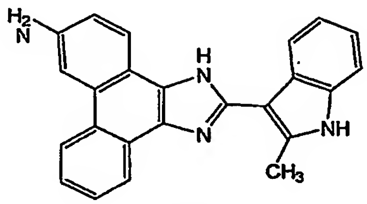

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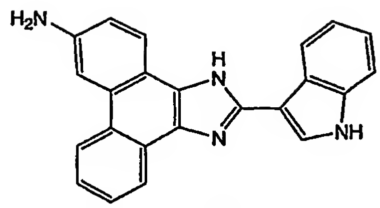

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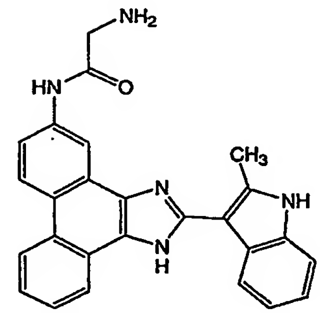

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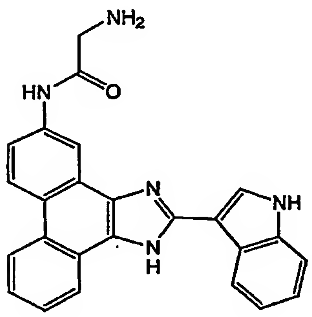

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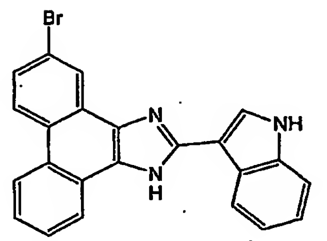

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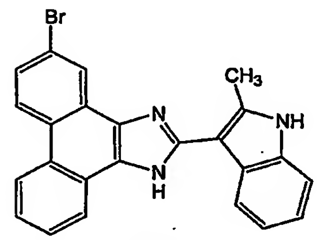

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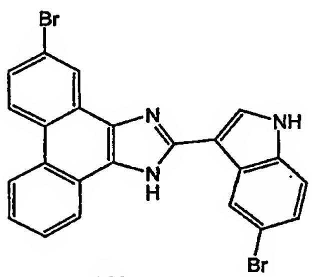

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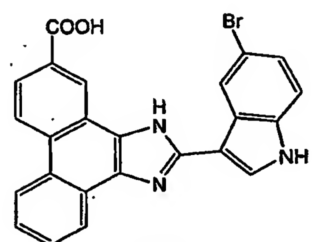

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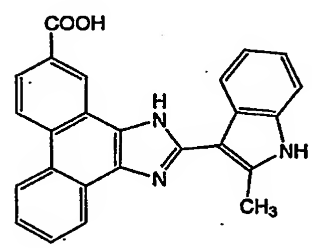

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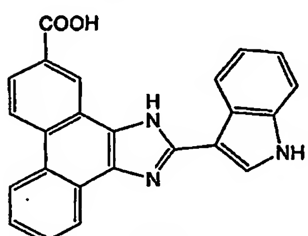

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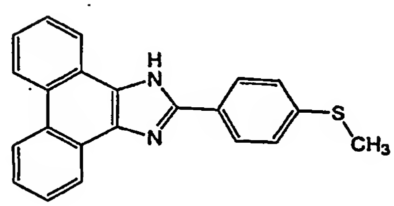

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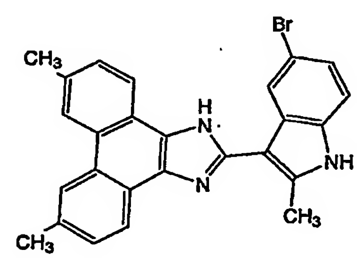

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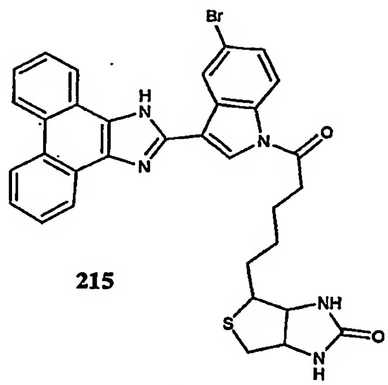

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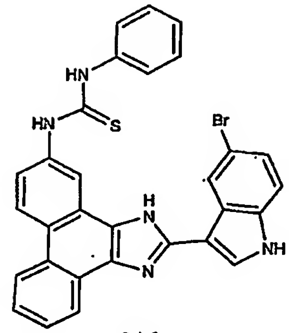

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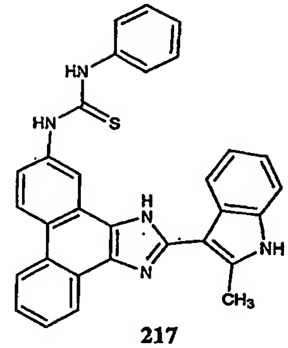

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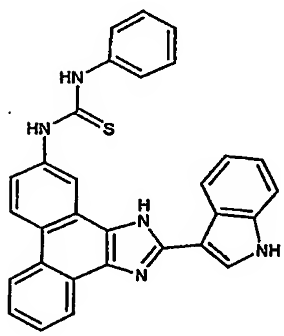

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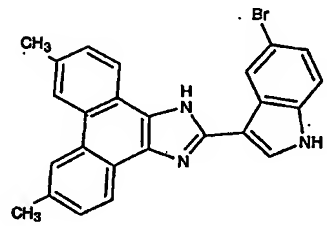

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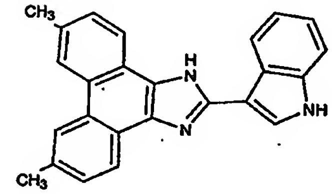

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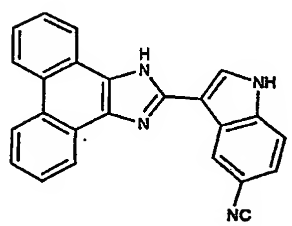

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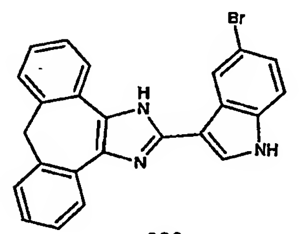

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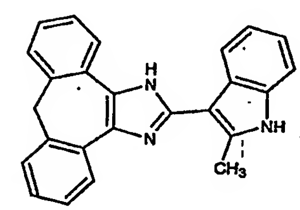

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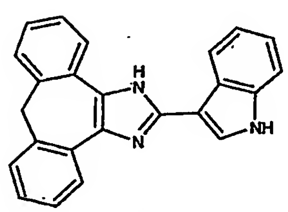

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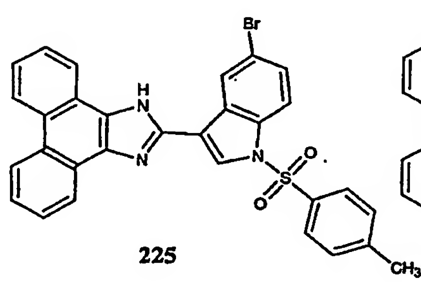

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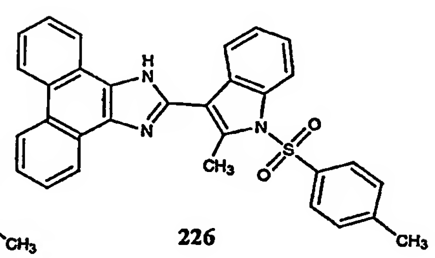

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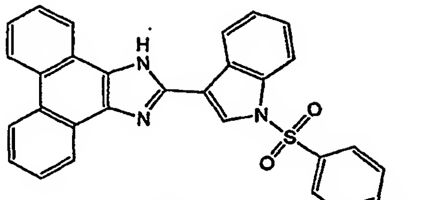

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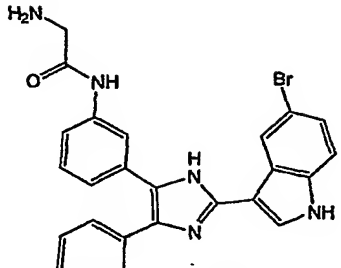

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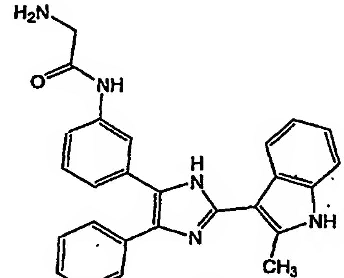

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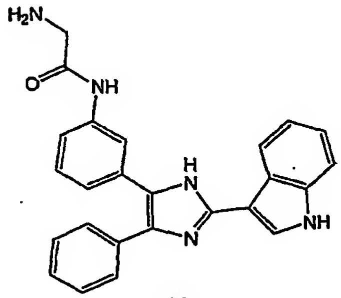

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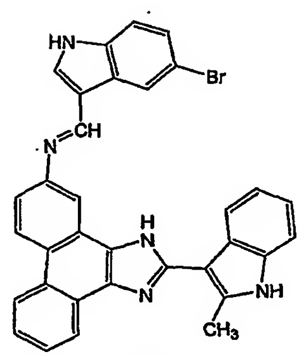

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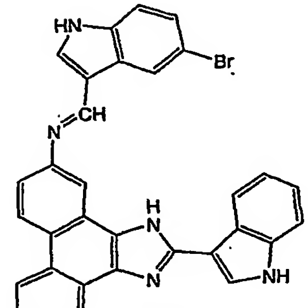

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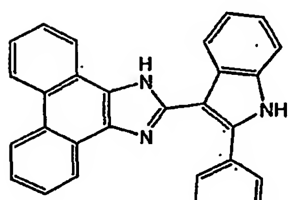

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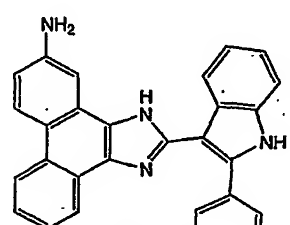

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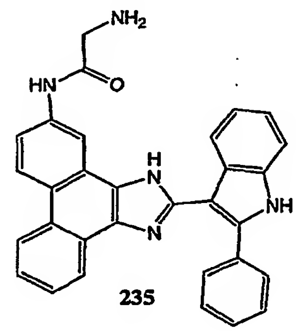

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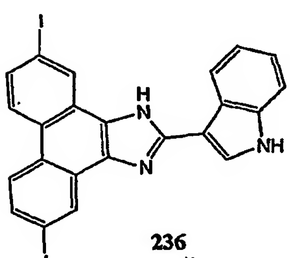

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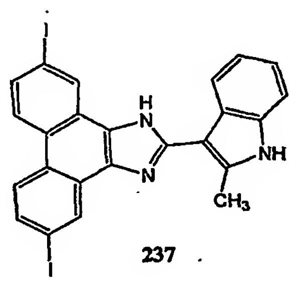

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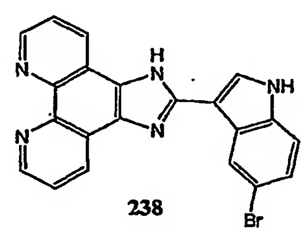

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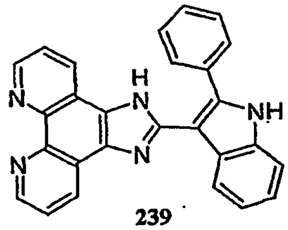
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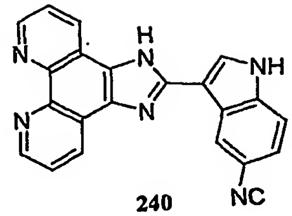
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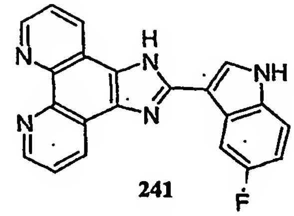
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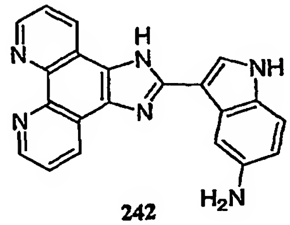
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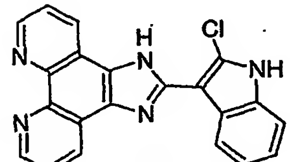
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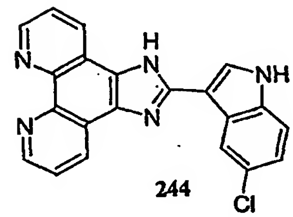
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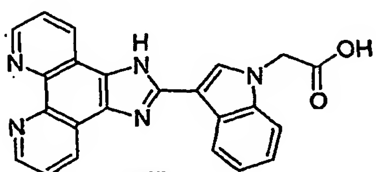
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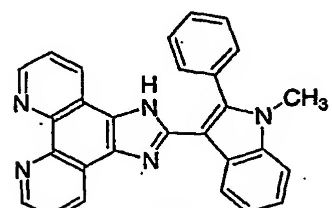
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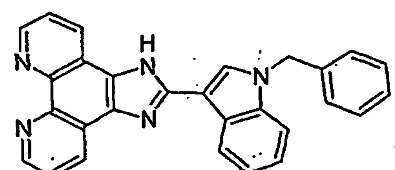
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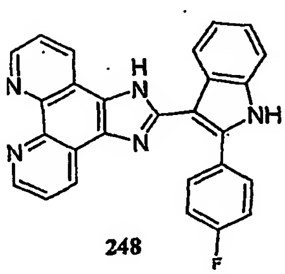
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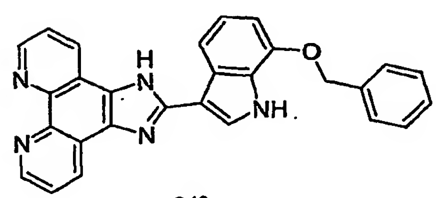
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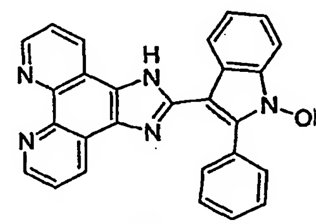
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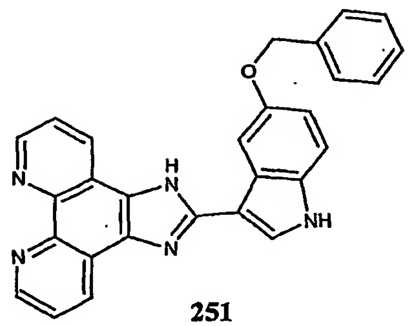

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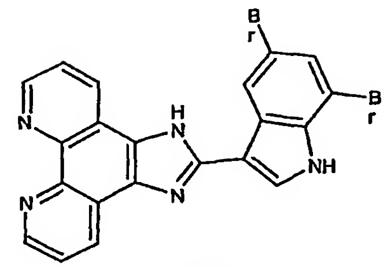

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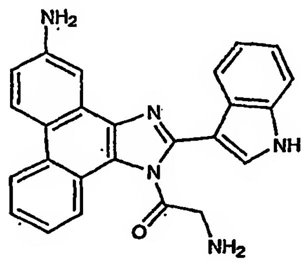

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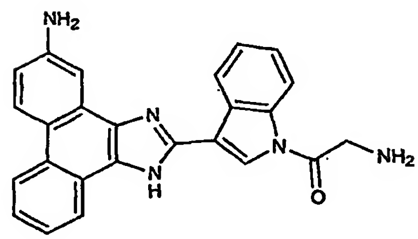

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The present invention includes pharmaceutically acceptable salts of the compounds defined by Formula I. Compounds according to the present invention can possess a sufficiently acidic, a sufficiently basic, or both functional groups, and accordingly react with a number of organic and inorganic bases, and organic and inorganic acids, to form pharmaceutically acceptable salts.

The term "pharmaceutically acceptable salt" as used herein, refers to a salt of a compound of Formula I, which is substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compound of the present invention with a pharmaceutically acceptable mineral or organic acid or an organic or inorganic base. Such salts are known as acid addition and base addition salts.

Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulphuric acid, phosphoric acid, and the like, and organic acids such as *p*-toluenesulphonic acid, methanesulphonic acid, oxalic acid, *p*-bromophenylsulphonic acid, carbonic acid, succinic acid, citric

acid, benzoic acid, acetic acid, and the like. Examples of such pharmaceutically acceptable salts are the sulphate, pyrosulphate, bisulphate, sulphite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, hydrochloride, dihydrochloride, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulphonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, gamma-hydroxybutyrate, glycolate, tartrate, methanesulphonate, propanesulphonate, naphthalene-1-sulfonate, napththalene-2-sulfonate, mandelate and the like. Preferred pharmaceutically acceptable acid addition salts are those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and those formed with organic acids such as maleic acid and methanesulphonic acid.

Salts of amine groups may also comprise quarternary ammonium salts in which the amino nitrogen carries a suitable organic group such as an alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, or aralkyl moiety.

Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like.

One skilled in the art will understand that the particular counterion forming a part of a salt of this invention is usually not of a critical nature, so long as the salt as a whole is pharmacologically acceptable and as long as the counterion does not contribute undesired qualities to the salt as a whole. The present invention further encompasses the pharmaceutically acceptable solvates of a compound of Formula I. Many of the compounds of Formula I can combine with solvents such as water, methanol, ethanol

and acetonitrile to form pharmaceutically acceptable solvates such as the corresponding hydrate, methanolate, ethanolate and acetonitrilate.

The compounds of the present invention may have multiple asymmetric (chiral) centres. As a consequence of these chiral centres, the compounds of the present invention occur as racemates, mixtures of enantiomers and as individual enantiomers, as well as diastereomers and mixtures of diastereomers. All asymmetric forms, individual isomers and combinations thereof, are within the scope of the present invention.

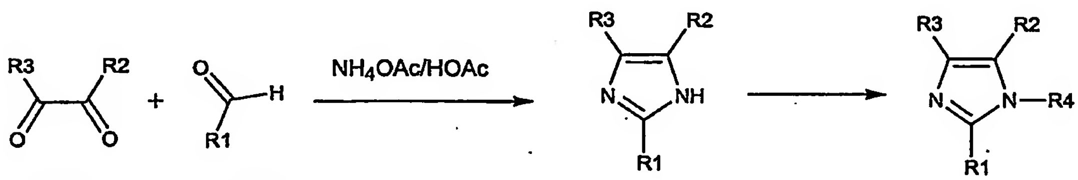
It will be readily understood by one skilled in the art that if the stereochemistry of a compound of Formula I is critical to its activity, then the relative stereochemistry of the compound is established early during synthesis to avoid subsequent stereoisomer separation problems. Further manipulation of the molecule will then employ stereospecific procedures so as to maintain the desired chirality.

Non-toxic metabolically-labile esters or amides of a compound of Formula I are those that are hydrolysed *in vivo* to afford the compound of Formula I and a pharmaceutically acceptable alcohol or amine. Examples of metabolically-labile esters include esters formed with (1-6C) alkanols, in which the alkanol moiety may be optionally substituted by a (1-8C) alkoxy group, for example methanol, ethanol, propanol and methoxyethanol. Non-limiting examples of metabolically-labile amides include amides formed with amines such as methylamine.

II. Preparation of Compounds of Formula I

As is known in the art, triaryl imidazole compounds can be prepared by a number of standard techniques. Compounds of Formula I, therefore, can be prepared by several general synthetic methods, for example, as described by Grimmett, (Grimmett, M.R., *Comprehensive Heterocyclic Chemistry: The Structure, Reaction, Synthesis and Uses of Heterocyclic Compounds*, A. R. Katrizky and C. W. Rees, eds., Vol. 5, Pergamon Press. Oxford, 1984, pp. 457-498; Grimmett, M. R., *Imidazole and Benzimidazole Synthesis*, Academic Press, San Diego CA, 1997).

In one embodiment of the present invention, compounds of Formula I are prepared *via* solution or solid phase synthesis, by reacting a dione of Formula II with the aldehyde (III) at elevated temperature in the presence of ammonium acetate in acetic acid (see, for example, Krieg *et al.*, *Naturforsch.* 1967, **22b**:132; Sarshar *et al.*, *Tetrahedron Lett.* 1996, **37**:835-838).

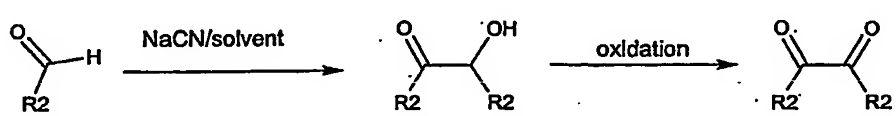


(XXXI) (XXXII)

The compounds of Formula (XXXI) and (XXXII) are either commercially available or may be prepared using standard procedures known to a person skilled in the relevant art. Compounds of Formula (XXXI), therefore, can be prepared by several general synthetic methods, for example, as described by: Fischer *et. al* (*J. Am. Chem. Soc.* **1961**, *83*, 4208-4210); Guijarro *et al.* (*J. Am. Chem. Soc.* **1999**, *121*, 4155-4157); Chi *et. al.* (*Synth. Comm.* **1994**, *24(15)*, 2119-2122) and Armesto *et. al.* (*Synthesis*, **1988**, 799-801).

Compounds of formula XXXI can also be prepared:

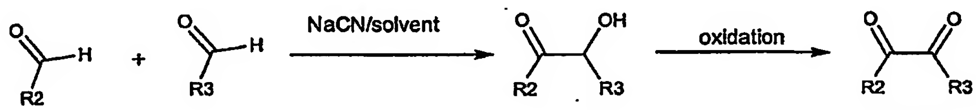
i) by oxidizing a compound of formula (XXXIII). Compounds of formula (XXXIII), in turn can be prepared by reacting a compounds of formula (XXXIV) with sodium cyanide in the presence of a solvent as shown below, wherein R3 = R2 and R2 is as defined above:



(XXXIV) (XXXIII) (XXXI)

or,

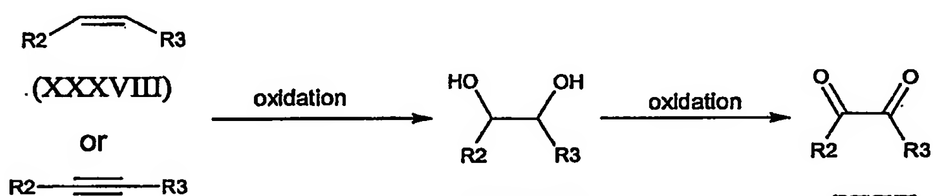
ii) by oxidizing a compound of formula (XXXV). Compounds of formula (XXXV), in turn can be prepared by treating a compound of formula (XXXIV) and a compound of formula (XXXVI) with sodium cyanide in the presence of a solvent as shown below, wherein R2 and R3 are as defined above:



(XXXIV) (XXXVI) (XXXV) (XXXI)

or,

iii) by oxidizing a compound of formula (XXXVII). Compounds of formula (XXXVII) in turn can be prepared by oxidizing a compound of formula (XXXVIII) or (XXXIX) as shown below, wherein R2 and R3 are as defined above:



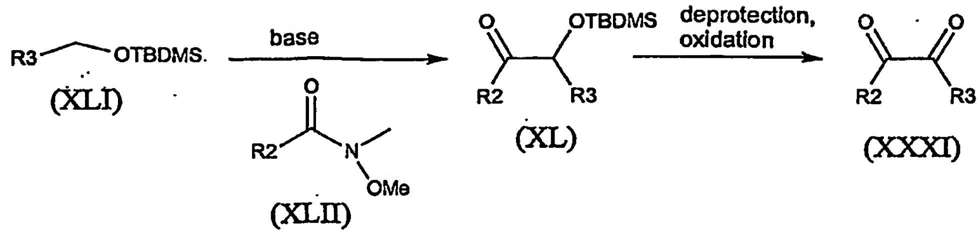
(XXXVIII) or (XXXIX) (XXXVII) (XXXI)

or,

iv) by oxidizing a compound of formula (XXXIX) using $PdCl_2$ in DMSO,

or,

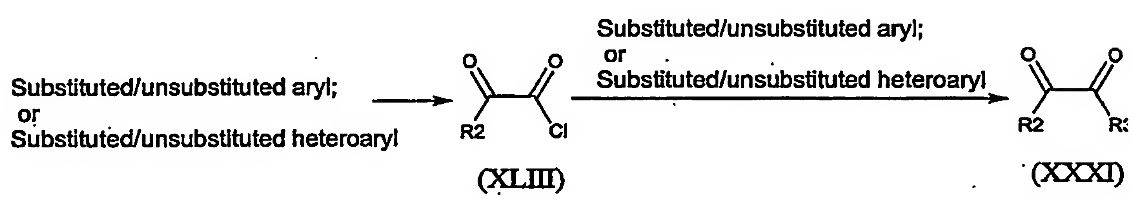
v) by deprotecting and oxidizing a compound of formula (XL). Compounds of formula (XL) in turn can be prepared by reacting a compound of formula (XLI) with a compound of formula (XLII) in the presence of a suitable base:



wherein R2 and R3 are independently aryl, substituted aryl, heteroaryl or substituted heteroaryl,

or,

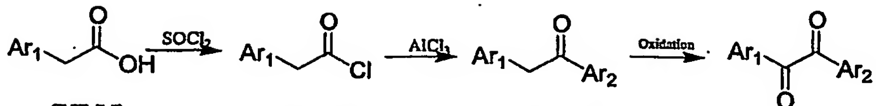
vi) by reacting a compound of formula (XLIII) with a substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl under Friedel-Crafts acylation conditions or by nucleophilic displacement of the chloride in compound of formula (XLIII). Compounds of formula (XLIII) in turn can be prepared by reacting a substituted or unsubstituted aryl or substituted heteroaryl or unsubstituted heteroaryl with oxalyl chloride under Friedel-Crafts acylation conditions:



wherein R2 and R3 are independently aryl, substituted aryl, heteroaryl or substituted heteroaryl;

or

vii) by oxidising a compound of formula (XLIV). Compounds of formula (XLIV) in turn can be prepared by reacting a compound of formula (XLV) with thionyl chloride in benzene with catalytic dimethylformamide to form an intermediate (XLVI). This intermediate (XLVI) is then used directly without purification in a Freidel–Crafts reaction to produce the ketone (XLIV).



(XLV) (XLVI) (XLIV) (XXXI)

III. Anti-cancer Activity of Compounds of Formula I

The ability of a candidate compound of Formula I to inhibit neoplastic cell growth and/or proliferation can be tested using standard techniques known in the art. In addition, compounds of Formula I that demonstrate inhibitory activity may be further tested *in vitro* and/or *in vivo* in combination with various known chemotherapeutics to evaluate their potential use in combination therapies. Exemplary methods of testing candidate compounds of Formula I are provided below and in the Examples included herein. One skilled in the art will understand that other methods of testing the compounds are known in the art and are also suitable for testing candidate compounds.

A. In vitro *Testing*

Candidate compounds of Formula I can be assayed initially *in vitro* for their ability to inhibit cell growth (*i.e.* their cytotoxicity) using standard techniques. In general, cells of a specific test cell line (typically a cancer cell line) are grown to a suitable density (*e.g.* approximately 1 x 10^4) and the candidate compound is added. After an appropriate incubation time (typically between about 48 to 74 hours), cell survival is assessed, for example, by assaying for tetrazolium salt (or modified tetrazolium salt) cleavage, or by using the resazurin reduction test (see Fields & Lancaster (1993) *Am. Biotechnol. Lab.* 11:48–50; O'Brien *et al.*, (2000) *Eur J. Biochem.* 267:5421-5426 and U.S. Patent No. 5,501,959), the sulforhodamine assay (Rubinstein *et al.*, (1990) *J. Natl. Cancer Inst.* 82:113-118) or the neutral red dye test (Kitano *et al.*, (1991) *Euro. J. Clin. Investg.* 21:53-58; West *et al.*, (1992) *J. Investigative Derm.* 99:95-100). Inhibition of cell growth is determined by comparison of cell survival in the treated culture with cell survival in one or more control cultures, for example, cultures not pre-treated with the candidate compound and/or those pre-treated with a control

compound (typically a known therapeutic). Other suitable techniques for assessing cytotoxicity are known in the art.

Assays that measure metabolic activity (such as tetrazolium-based assays) can also be used to assess the effect of candidate compounds on cell activation and /or proliferation, due the fact that proliferating cells are metabolically more active than resting cells.

Candidate compounds can also be tested *in vitro* for their ability to inhibit anchorage-independent growth of tumour cells. Anchorage-independent growth is known in the art to be a good indicator of tumourigenicity. In general, anchorage-independent growth is assessed by plating cells from an appropriate cancer cell-line onto soft agar and determining the number of colonies formed after an appropriate incubation period. Growth of cells treated with the candidate compound can then be compared with that of cells treated with an appropriate control (as described above).

A variety of cancer cell-lines suitable for testing the candidate compounds are known in the art. In one embodiment of the present invention, *in vitro* testing of the candidate compounds is conducted in a human cancer cell-line. Examples of suitable human cancer cell-lines for *in vitro* testing of the compounds of the present invention include, but are not limited to, colon and colorectal carcinoma cell lines such as HT-29, CaCo, LoVo, COLO320 and HCT-116; non small cell lung cancer cell lines such as NCI-H460, small cell lung cancer cell lines such as H209; breast cancer cell lines such as MCF-7, T47D and MDA-MB-231; ovarian cancer cell lines such as SK-OV-3; prostate cancer cell lines such as PC-3 and DU-145; chronic myeloid leukaemia cell lines such as K562; bladder cancer cell lines such as T24; brain cancer cell lines such as U-87-MG; pancreatic cancer cell lines such as AsPC-1, SU.86.86 and BxPC-3; kidney cancer cell lines such as A498 and Caki-1; liver cancer cell lines such as HepG2, and skin cancer cell lines such as A2058 and C8161. Drug-resistant cancer cell lines can be used to determine the ability of the compounds of the present invention to inhibit growth and/or proliferation of drug- or multi-drug resistant neoplastic cells. .

The selectivity of the candidate compounds of Formula I may also be tested, *i.e.* the ability of the compound to demonstrate some level of selective action toward neoplastic (or cancer) cells in comparison to normal proliferating cells. An exemplary method of assessing the differential sensitivity between normal and cancer cells for a compound has been described by Vassilev *et al.* (*Anti-Cancer Drug Design* (2001) 16:7). This method involves the comparison of IC_{90} values, *i.e.* the molar concentration of a test compound required to cause 90% growth inhibition of exponentially growing cells. Thus, the IC_{90} values for candidate compounds can be evaluated in various cancer cell lines (such as those outlined above) and normal cells (such as HUVEC and/or WI38 cells) and compared. IC_{90} values can be measured using a variety of standard techniques including those described above for cytotoxicity testing.

While the mechanism of action of the compounds of Formula I is not relevant to the instant invention, assays to investigate potential mechanisms of action of the compounds may be conducted if desired in order to provide information useful in determining what aspects of tumour growth the compounds affect. This type of information may help to determine cancer types that will benefit from treatment with the compounds. Examples of such assays include, but are not limited to, cell-cycle analysis (for example, employing flow cytometry techniques), apoptosis assays (such as DNA fragmentation analysis), anti-angiogenesis assays (for example, various Matrigel assays, including cord formation and Matrigel plug assays) and immunohistochemical analysis.

Toxicity of the candidate compounds can also be initially assessed *in vitro* using standard techniques. For example, human primary fibroblasts can be treated *in vitro* with a compound of Formula I and then tested at different time points following treatment for their viability using a standard viability assay, such as the assays described above or the trypan-blue exclusion assay. Cells can also be assayed for their ability to synthesize DNA, for example, using a thymidine incorporation assay, and for changes in cell cycle dynamics, for example, using a standard cell sorting assay in conjunction with a fluorocytometer cell sorter (FACS).

B. In vivo *Testing*

The ability of the candidate compounds to inhibit tumour growth, proliferation and/or metastasis *in vivo* can be determined in an appropriate animal model using standard techniques known in the art (see, for example, Enna, *et al., Current Protocols in Pharmacology,* J. Wiley & Sons, Inc., New York, NY). Exemplary protocols are provided below and in the Examples. Non-limiting examples of suitable animal models are provided in Table 1.

In general, current animal models for screening anti-tumour compounds are xenograft models, in which a human tumour has been implanted into an animal. For example, the candidate compounds can be tested *in vivo* on solid tumours using mice that are subcutaneously grafted or injected with 30 to 60 mg of a tumour fragment, or an appropriate number of tumour cells (*e.g.* about 10^6 to 10^7) on day 0. The animals bearing tumours are mixed before being subjected to the various treatments and controls. In the case of treatment of advanced tumours, tumours are allowed to develop to the desired size, animals having insufficiently developed tumours being eliminated. The selected animals are distributed at random to undergo the treatments and controls. Animals not bearing tumours may also be subjected to the same treatments as the tumour-bearing animals in order to be able to dissociate the toxic effect from the specific effect on the tumour. Chemotherapy generally begins from 3 to 22 days after grafting, depending on the type of tumour, and the animals are observed every day. Candidate compounds can be administered to the animals, for example, by bolus infusion. The different animal groups are weighed about 3 or 4 times a week until the maximum weight loss is attained, after which the groups are weighed at least once a week until the end of the trial.

The tumours are measured about 2 or 3 times a week until the tumour reaches a pre-determined size and / or weight, or until a pre-determined time period has passed, or until the animal dies (if this occurs before the tumour reaches the pre-determined size / weight). The animals are then sacrificed and the tissue histology, size and / or proliferation of the tumour assessed.

If desired, one or more standard immunohistochemical tests may also be conducted on tissues isolated from the test animals in order to determine the effects of the compound on tumour growth, differentiation, apoptosis and/or angiogenesis. Examples of such tests include, but are not limited to, the use of specific antibodies (for example, antibodies against Ki-67 to assess proliferation, CD31 to assess angiogenesis, NK1.1 as an indication of the presence of NK cells, F4/80 as an indication of the presence of macrophages) and TUNEL assays to determine apoptosis.

Other models, such as orthopedic implantation of tumours into animals (*i.e.* the implantation of cancer cells of a certain type into the corresponding tissue in the animal, such as pancreatic cancer cells into the pancreas), may also be used to assess the effect of the candidate compounds on tumour growth and proliferation. In addition, the effect of the candidate compound on spontaneous tumours in normal mice can be assessed.

The effect of the candidate compounds on drug-resistant tumours can be assessed *in vivo* by utilising a drug- or multidrug-resistant cancer cell in the xenograft experiments.

For the study of the effect of the candidate compounds on haematologic tumours, such as lymphomas or leukaemias, the animals are grafted or injected with a particular number of cells, and the anti-tumour activity is determined by the increase in the survival time of the treated mice relative to the controls.

To study the effect of the candidate compounds on tumour metastasis, tumour cells are typically treated with the compound *ex vivo* and then injected into a suitable test animal. The spread of the tumour cells from the site of injection is then monitored over a suitable period of time.

The ability of the candidate compounds to act in combination with, or to sensitise a tumour to the effects of, another chemotherapeutic agent can also be tested in the above models. In this case, the test animals would be treated with both the chemotherapeutic agent and the candidate compound of Formula I. Control animals

could include animals treated with the chemotherapeutic alone, animals treated with the candidate compound alone and/or untreated animals.

In vivo toxic effects of the compounds of Formula I can be evaluated by standard techniques, for example, by measuring their effect on animal body weight during treatment and by performing haematological profiles and liver enzyme analysis after the animal has been sacrificed (survival assays).

Table I: Examples of *in vivo* models of human cancer

Cancer Model	Cell Type
Tumour Growth Assay Human solid tumour xenografts in mice (sub-cutaneous injection)	Prostate (PC-3, DU145) Breast (MDA-MB-231, MVB-9) Colon (HT-29) Lung (NCI-H460, NCI-H209) Pancreatic (ASPC-1, SU86.86) Pancreatic: drug resistant (BxPC-3) Skin (A2058, C8161) Cervical (SIHA, HeLa-S3) Cervical: drug resistant (HeLa S3-HU-resistance) Liver (HepG2) Brain (U87-MG) Renal (Caki-1, A498) Ovary (SK-OV-3) Bladder (T24)
Tumour Growth Assay Human solid tumour isografts in mice (fat pad injection)	Breast: drug resistant (MDA-CDDP-S4, MDA-MB435-To.1)
Survival Assay Experimental model of lymphoma and leukaemia in mice	Human: Burkitts lymphoma (Non-Hodgkin's) (raji) Murine: erythroleukemia (CB7 Friend retrovirus-induced)
Experimental model of lung metastasis in mice	Human: melanoma (C8161) Murine: fibrosarcoma (R3)

IV. Toxicity Testing

It is important that the anti-cancer compounds of the present invention exhibit low toxicity *in vivo*. Toxicity tests for potential drugs are well-known in the art (see, for example, Hayes, A.W., ed., (1994), *Principles and Methods of Toxicology,* 3rd ed., Raven Press, NY; Maines, M., ed., *Current Protocols in Toxicology,* John Wiley & Sons, Inc., NY).

In vitro acute toxicity testing of a compound of Formula I can be performed using mammalian cell lines (see, for example, Ekwall, B., *Ann. N.Y. Acad. Sci.,* (1983) 407:64-77). Selection of an appropriate cell line is dependent on the potential application of the candidate compound and can be readily determined by one skilled in the art.

In vivo toxicity testing can be performed by standard methodology. For example, by injecting varying concentrations of the candidate compound into an appropriate animal model. The compound can be injected once, or administration can be repeated over several days. The toxic effects of the compound can be evaluated over an appropriate time period by monitoring the general health and body weight of the animals. After the completion of the period of assessment, the animals can be sacrificed and the appearance and weight of the relevant organs determined. An indication of the toxicity of a compound can also be obtained during the *in vivo* anti-cancer testing of the compound.

V. Therapeutic Uses of Compounds of Formula I

The compounds of Formula I can be used in the treatment and/or stabilisation of various types of cancers. In this context, the compounds may exert either a cytotoxic or cytostatic effect resulting in a reduction in the size of a tumour, the slowing or prevention of an increase in the size of a tumour, an increase in the disease-free survival time between the disappearance or removal of a tumour and its reappearance, prevention of an initial or subsequent occurrence of a tumour (*e.g.* metastasis), an

increase in the time to progression, reduction of one or more adverse symptom associated with a tumour, or an increase in the overall survival time of a subject having cancer. The compounds can be used alone or they can be used as part of a multi-drug regimen in combination with one or more known therapeutics.

Examples of cancers which may be may be treated or stabilized in accordance with the present invention include, but are not limited to haematologic neoplasms, including leukaemias and lymphomas; carcinomas, including adenocarcinomas; melanomas and sarcomas. Carcinomas, adenocarcinomas and sarcomas are also frequently referred to as "solid tumours." Examples of commonly occurring solid tumours include, but are not limited to, cancer of the brain, breast, cervix, colon, head and neck, kidney, lung, ovary, pancreas, prostate, stomach and uterus, non-small cell lung cancer and colorectal cancer. Various forms of lymphoma also may result in the formation of a solid tumour and, therefore, are also often considered to be solid tumours. One embodiment of the present invention provides for the use of the compounds of Formula I in the treatment and/or stabilisation of a solid tumour.

The term "leukaemia" refers broadly to progressive, malignant diseases of the blood-forming organs. Leukaemia is typically characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow but can also refer to malignant diseases of other blood cells such as erythroleukaemia, which affects immature red blood cells. Leukaemia is generally clinically classified on the basis of (1) the duration and character of the disease – acute or chronic; (2) the type of cell involved – myeloid (myelogenous), lymphoid (lymphogenous) or monocytic, and (3) the increase or non-increase in the number of abnormal cells in the blood – leukaemic or aleukaemic (subleukaemic). Leukaemia includes, for example, acute nonlymphocytic leukaemia, chronic lymphocytic leukaemia, acute granulocytic leukaemia, chronic granulocytic leukaemia, acute promyelocytic leukaemia, adult T-cell leukaemia, aleukaemic leukaemia, aleukocythemic leukaemia, basophylic leukaemia, blast cell leukaemia, bovine leukaemia, chronic myelocytic leukaemia, leukaemia cutis, embryonal leukaemia, eosinophilic leukaemia, Gross' leukaemia, hairy-cell leukaemia, hemoblastic leukaemia, hemocytoblastic leukaemia, histiocytic leukaemia, stem cell leukaemia, acute monocytic leukaemia, leukopenic leukaemia,

lymphatic leukaemia, lymphoblastic leukaemia, lymphocytic leukaemia, lymphogenous leukaemia, lymphoid leukaemia, lymphosarcoma cell leukaemia, mast cell leukaemia, megakaryocytic leukaemia, micromyeloblastic leukaemia, monocytic leukaemia, myeloblastic leukaemia, myelocytic leukaemia, myeloid granulocytic leukaemia, myelomonocytic leukaemia, Naegeli leukaemia, plasma cell leukaemia, plasmacytic leukaemia, promyelocytic leukaemia, Rieder cell leukaemia, Schilling's leukaemia, stem cell leukaemia, subleukaemic leukaemia, and undifferentiated cell leukaemia.

The term "sarcoma" generally refers to a tumour which originates in connective tissue, such as muscle, bone, cartilage or fat, and is made up of a substance like embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas include soft tissue sarcomas, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumour sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented haemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma.

The term "melanoma" is taken to mean a tumour arising from the melanocytic system of the skin and other organs. Melanomas include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, and superficial spreading melanoma.

The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas include, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colorectal carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, haematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal carcinoma, oat cell carcinoma, non-small cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional

cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum.

The term "carcinoma" also encompasses adenocarcinomas. Adenocarcinomas are carcinomas that originate in cells that make organs which have glandular (secretory) properties or that originate in cells that line hollow viscera, such as the gastrointestinal tract or bronchial epithelia. Examples include, but are not limited to, adenocarcinomas of the breast, lung, colon, pancreas and prostate.

Additional cancers encompassed by the present invention include, for example, Hodgkin's Disease, Non-Hodgkin's lymphoma, multiple myeloma, neuroblastoma, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumours, primary brain tumours, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, gliomas, testicular cancer, thyroid cancer, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, mesothelioma and medulloblastoma.

The cancer to be treated or stabilized may be indolent or it may be aggressive. The compounds of the invention can be used to treat refractory cancers, advanced cancers, recurrent cancers and metastatic cancers. One skilled in the art will appreciate that many of these categories may overlap, for example, aggressive cancers are typically also metastatic.

"Aggressive cancer," as used herein, refers to a rapidly growing cancer. One skilled in the art will appreciate that for some cancers, such as breast cancer or prostate cancer the term "aggressive cancer" will refer to an advanced cancer that has relapsed within approximately the earlier two-thirds of the spectrum of relapse times for a given cancer, whereas for other types of cancer, such as small cell lung carcinoma (SCLC), nearly all cases present rapidly growing cancers which are considered to be aggressive. The term can thus cover a subsection of a certain cancer type or it may encompass all of other cancer types. A "refractory" cancer or tumour refers to a cancer or tumour that has not responded to treatment. "Advanced cancer," refers to overt disease in a patient that is not amenable to cure by local modalities of treatment,

such as surgery or radiotherapy. Advanced disease may refer to a locally advanced cancer or it may refer to metastatic cancer. The term "metastatic cancer" refers to cancer that has spread from one part of the body to another.

The present invention also contemplates the use of the compounds of Formula I as "sensitizing agents," which selectively inhibit the growth of cancer cells. In this case, the compound alone does not have a cytotoxic effect on the cancer cell, but provides a means of weakening the cancer cells, and thereby facilitates the benefit from conventional anti-cancer therapeutics.

Thus, the present invention contemplates the administration to a subject of a therapeutically effective amount of one or more compound of Formula I together with one or more anti-cancer therapeutics. The compound(s) can be administered before, during or after treatment with the anti-cancer therapeutic. An "anti-cancer therapeutic" is a compound, composition or treatment that prevents or delays the growth and/or metastasis of cancer cells. Such anti-cancer therapeutics include, but are not limited to, chemotherapeutic drug treatment, radiation, gene therapy, hormonal manipulation, immunotherapy and antisense oligonucleotide therapy. A wide variety of chemotherapeutic drugs are known in the art and can be used in combination therapies with a compound of the present invention. Examples of useful chemotherapeutic drugs include broad spectrum chemotherapeutics, *i.e.* those that are useful in the treatment of a range of cancers, such as doxorubicin, capecitabine, mitoxantrone, irinotecan (CPT-11), cisplatin and gemcitabine. Other examples of useful chemotherapeutic agents include, but are not limited to, hydroxyurea, busulphan, carboplatin, chlorambucil, melphalan, cyclophosphamide, Ifosphamide, danorubicin, epirubicin, vincristine, vinblastine, Navelbine® (vinorelbine), etoposide, teniposide, paclitaxel, docetaxel, cytosine, arabinoside, bleomycin, neocarcinostatin, suramin, taxol, mitomycin C and the like. The compounds of the invention are also suitable for use with standard combination therapies employing two or more chemotherapeutic agents. It is to be understood that anti-cancer therapeutics for use in the present invention also include novel compounds or treatments developed in the future.

VI. Pharmaceutical Compositions

The compounds of the present invention are typically formulated prior to administration. The present invention thus provides pharmaceutical compositions comprising one or more compounds of Formula I and a pharmaceutically acceptable carrier, diluent, or excipient. The pharmaceutical compositions are prepared by known procedures using well-known and readily available ingredients. Pharmaceutical compositions comprising one or more compounds of Formula I in combination with one or more known cancer chemotherapeutics are also contemplated by the present invention.

Compounds of the general Formula I or pharmaceutical compositions comprising the compounds may be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. In the usual course of therapy, the active compound is incorporated into an acceptable vehicle to form a composition for topical administration to the affected area, such as hydropohobic or hydrophilic creams or lotions, or into a form suitable for oral, rectal or parenteral administration, such as syrups, elixirs, tablets, troches, lozenges, hard or soft capsules, pills, suppositiories, oily or aqueous suspensions, dispersible powders or granules, emulsions, injectables, or solutions. The term parenteral as used herein includes subcutaneous injections, intradermal, intra-articular, intravenous, intramuscular, intravascular, intrasternal, intrathecal injection or infusion techniques.

Compositions intended for oral use may be prepared in either solid or fluid unit dosage forms. Fluid unit dosage form can be prepared according to procedures known in the art for the manufacture of pharmaceutical compositions and such compositions may contain ~~one or more agents~~ selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. An elixir is prepared by using a hydroalcoholic (e.g., ethanol) vehicle with suitable sweeteners such as sugar and saccharin, together with an aromatic flavoring agent. Suspensions can be

prepared with an aqueous vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

Solid formulations such as tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate: granulating and disintegrating agents for example, corn starch, or alginic acid: binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc and other conventional ingredients such as dicalcium phosphate, magnesium aluminum silicate, calcium sulfate, starch, lactose, methylcellulose, and functionally similar materials. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil. Soft gelatin capsules are prepared by machine encapsulation of a slurry of the compound with an acceptable vegetable oil, light liquid petrolatum or other inert oil.

Aqueous suspensions contain active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxylmethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia: dispersing or wetting agents may be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example hepta-decaethyleneoxycetanol, or condensation products of ethylene oxide with partial

esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or *n*-propyl- *p*-hydroxy benzoate, one or more colouring agents, one or more flavouring agents or one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example peanut oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, may also be present.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oil phase may be a vegetable oil, for example olive oil or peanut oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or a suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Adjuvants such as local anaesthetics, preservatives and buffering agents can also be included in the injectable solution or suspension.

The compound(s) of the general Formula I may be administered, together or separately, in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Other pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "*Remington: The Science and Practice of Pharmacy*" (formerly "*Remingtons Pharmaceutical Sciences*"); Gennaro, A., Lippincott, Williams & Wilkins, Philidelphia, PA (2000).

VII. Administration of Compounds of Formula I

Compounds of Formula I may be administered to a subject by a variety of routes depending on the cancer to be treated, for example, the compounds may be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations. In one embodiment, the compounds are administered systemically to a subject, for example, by bolus injection or continuous infusion into a

subject's bloodstream or by oral administration. When used in conjunction with one or more known chemotherapeutic agents, the compounds can be administered prior to, or after, administration of the chemotherapeutic agents, or they can be administered concomitantly. The one or more chemotherapeutic may also be administered systemically, for example, by bolus injection, continuous infusion, or oral administration.

The compounds of Formula I may be used as part of a neo-adjuvant therapy (to primary therapy), or as part of an adjuvant therapy regimen, where the intention is to cure the cancer in a subject. The present invention contemplates the use of the compounds of Formula I at various stages in tumour development and progression, including in the treatment of advanced and/or aggressive neoplasias (*i.e.* overt disease in a subject that is not amenable to cure by local modalities of treatment, such as surgery or radiotherapy), metastatic disease, locally advanced disease and/or refractory tumours (*i.e.* a cancer or tumour that has not responded to treatment).

"Primary therapy" refers to a first line of treatment upon the initial diagnosis of cancer in a subject. Exemplary primary therapies may involve surgery, a wide range of chemotherapies and radiotherapy. "Adjuvant therapy" refers to a therapy that follows a primary therapy and that is administered to subjects at risk of relapsing. Adjuvant systemic therapy is usually begun soon after primary therapy to delay recurrence, prolong survival or cure a subject.

It is contemplated that the compounds of the invention can be used alone or in combination with one or more other chemotherapeutic agents as part of a primary therapy or an adjuvant therapy. Combinations of the compounds of Formula I and standard chemotherapeutics may act to improve the efficacy of the chemotherapeutic and, therefore, can be used to improve standard cancer therapies. This application can be important in the treatment of drug-resistant cancers which are not responsive to standard treatment. Drug-resistant cancers can arise, for example, from heterogeneity of tumour cell populations, alterations in response to chemotherapy and increased malignant potential. Such changes are often more pronounced at advanced stages of disease.

The dosage to be administered is not subject to defined limits, but it will usually be an effective amount. It will usually be the equivalent, on a molar basis of the pharmacologically active free form produced from a dosage formulation upon the metabolic release of the active free drug to achieve its desired pharmacological and physiological effects. The compositions may be formulated in a unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Examples of ranges for the compound(s) in each dosage unit are from about 0.05 to about 100 mg, or more usually, from about 1.0 to about 50 mg.

Daily dosages of the compounds of the present invention will typically fall within the range of about 0.01 to about 100 mg/kg of body weight, in single or divided dose. However, it will be understood that the actual amount of the compound(s) to be administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms. The above dosage range is given by way of example only and is not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing harmful side effects, for example, by first dividing the larger dose into several smaller doses for administration throughout the day.

VIII. Kits

The present invention additionally provides for therapeutic kits containing one or more compounds of Formula I for use in the treatment of cancer. The contents of the kit can be lyophilized and the kit can additionally contain a suitable solvent for reconstitution of the lyophilized components. Individual components of the kit would be packaged in separate containers and, associated with such containers, can be a notice in the form prescribed by a governmental agency regulating the manufacture,

use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, for use or sale for human or animal administration.

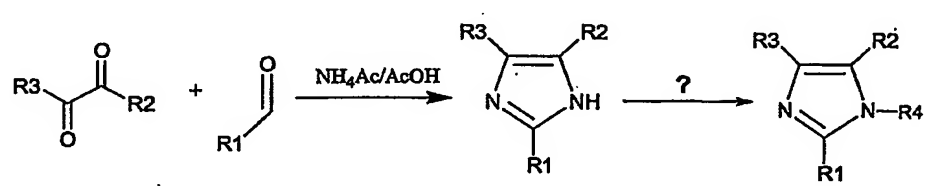
When the components of the kit are provided in one or more liquid solutions, the liquid solution can be an aqueous solution, for example a sterile aqueous solution. For *in vivo* use, the compounds may be formulated into a pharmaceutically acceptable syringeable composition. In this case the container means may itself be an inhalant, syringe, pipette, eye dropper, or other such like apparatus, from which the formulation may be applied to an infected area of the subject, such as the lungs, injected into an subject, or even applied to and mixed with the other components of the kit.

Pharmaceutical kits or packs comprising one or more compound of the present invention in combination with one or more standard chemotherapeutic for combination therapy applications are also contemplated by the present invention.
To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only. Therefore, they should not limit the scope of this invention in any way.

EXAMPLES

Preparation of compounds:

All reactions have been carried out according to the scheme shown below:

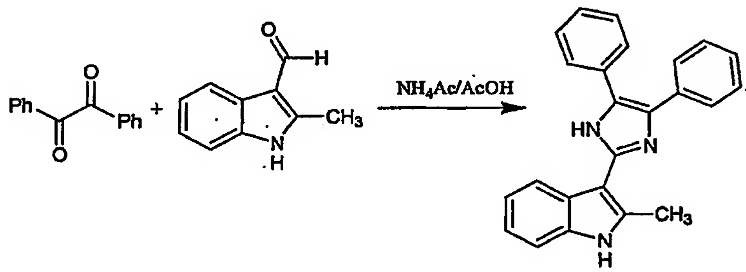


In a typical experimental procedure 1 mmol (1 equiv.) of the carboxyaldehyde was combined with 1.05 - 1.10 mmole (1.05 - 1.1 equiv.) of the dione and 20 mmole (20 equiv.) of ammonium acetate and 5 ml of acetic acid. The mixture was magnetically stirred and heated to reflux for 3-5 hr. The reaction process was monitored by TLC,

until complete consumption of the indole was achieved. The reaction mixture was cooled to room temperature and added drop-wise into well-stirred ice-water. The suspension solid was then filtered and the crude solid was dissolved in ethyl acetate, dried over sodium sulfate and filtered, the organic solvent was removed by vacuum. The products was then either recrystalized with alcohol or separated by column chromatography using petroleum ether-Ethyl acetate as an eluant.

Melting points were recorded using a MEL-TEMP capillary melting point apparatus, the melting point are uncorrected. ^{1}H-NMR was performed in a 500 MHz Brucker instrument at room temperature using a suitable deuterated solvent.

Example 1: Preparation of compound 2



2

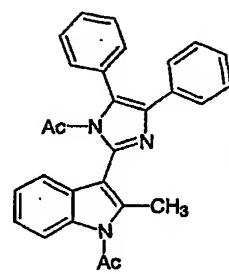
1 mmol (1 equiv.) of the indole carboxyaldehyde was combined with 1.05 - 1.10 mmole (1.05 - 1.1 equiv.) of the benzil and 20 mmole (20 equiv.) of ammonium acetate and 5 ml of acetic acid. The mixture was magnetically stirred and heated to reflux for 3-5 hr. The reaction process was monitored by TLC, until complete consumption of the indole was achieved. The reaction mixture was cooled to room temperature and added drop-wise into well-stirred ice-water. The suspension solid was then filtered and the crude solid was ~~dissolved~~ in ethyl acetate, dried over sodium sulfate and filtered, the organic solvent was removed by vacuum. The products was then either recrystalized with alcohol or separated by column chromatography using petroleum ether-Ethyl acetate as an eluant.

It is noteworthy that, the TLC of the products shows a characteristic blue florescent color under the UV (wave length λ = 254nm), a property used as an additional characterization feature.

^{1}H-NMR: δ (DMSO-d_6), 12.10 (s, 1H), 11.30 (s, 1H), 7.98 (d, 1H), 7.62 (d, 2H), 7.56 (d, 2H), 7.45 (t, 2H), 7.28-7.40 (m, 4H), 7.24 (t, 1H), 7.03-7.14 (m, 2H), 2.70 (s, 3H). HRMS m/z for $C_{24}H_{19}N_3$ calc. Is 349.157898, found 349.157897. M.p.= decomposed at 260-264.

The following exemplary compounds were also prepared from the appropriate starting materials following the general synthetic procedure as discussed above.

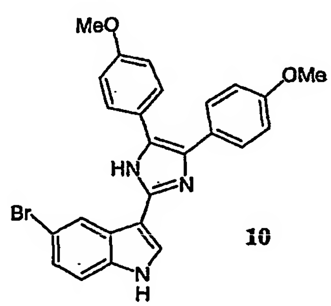
Example 2: Compound 5



5

^{1}H-NMR ($CDCl_3$): δ = 8.02 (d, 2H), 7.53 (d, 1H), 7.43 - 7.52 (m, 6H), 7.41 (d, 1H), 7.21 - 7.34 (m, 6H), 2.81 (s, 3H), 2.75 (s, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{28}H_{23}N_3O_2$ is 433. M.p.= 224-227.

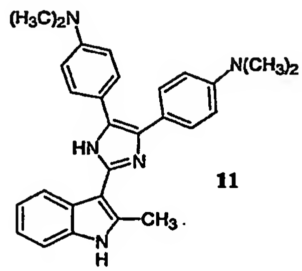
Example 3: Compound 10



10

^{1}H-NMR ($CDCl_3$): δ = 10.68 (bs, 1H), 7.73 (bs, 1H), 7.22 (d, 4H), 6.99 (bs, 1H), 6.92 (bd, 2H), 6.85 (bd, 2H), 6.611 (d, 4H), 3.70 (s, 6H). EIMS [$M^{+\cdot}$] m/z for $C_{25}H_{20}N_3BrO_2$ is 474. M.p.= 135.

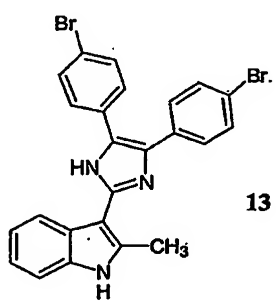
Example 4: Compound 11



11

^{1}H-NMR ($CDCl_3$): δ = 7.70 (d, 1H), 7.41 (d, 4H), 7.32 (d, 1H), 7.09 (q, 2H), 6.77 (d, 4H), 2.95 (s, 12H), 2.67 (s, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{28}H_{29}N_5$ is 435, M.p.= decomposed at 236-238.

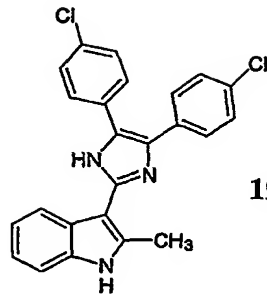
Example 5: Compound 13



13

^{1}H-NMR ($CDCl_3$): δ = 7.47 (d, 4H), 7.44 (d, 4H), 7.30-7.34 (m, 1H), 7.14 - 7.19 (m, 3H), 2.68 (bs, 3H), EIMS [$M^{+\cdot}$] m/z for $C_{24}H_{17}N_3Br_2$ is 507. M.p.= 240-245.

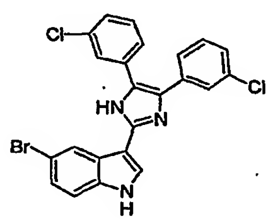
Example 6: Compound 19



19

^{1}H-NMR (DMSO-d$_6$): δ = 12.13 (s, 1H), 11.33 (s, 1H), 7.94 (d, 2H), 7.57 (d, 2H), 7.52 (bd, 2H), 7.39 (bd, 2H), 7.35 (d, 1H), 7.05-7.12 (m, 3H), 2.50 (s, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{24}H_{17}N_3Cl_2$ is 418. M.p.= 165-167.

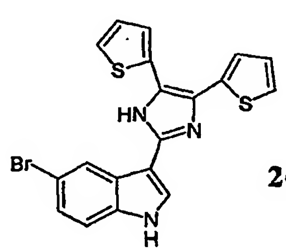
Example 7: Compound 22



22

^{1}H-NMR (DMSO-d$_6$): δ =13.176 (s) 1H, 12.130 (s) 1H, 8.996 (d) 1H, 8.889 (d) 1H, 8.852 (d) 1H, 8.671 (d) 1H 8.412 (d) 1H, 8.378 (d) 1H, 7.775-7.750 (m) 2H, 7.640-7.600 (m) 2H.

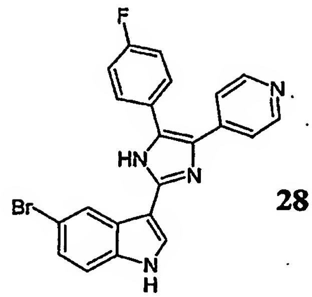
Example 8: Compound 26



26

^{1}H-NMR (DMSO-d$_6$): δ = 12.60 (s, 1H), 11.70 (s, 1H), 8.60 (d, 1H), 8.17 (s, 1H), 7.68 (bs, 1H), 7.46 (d, 2H), 7.33 (d, 2H), 7.25 (bs, 2H), 7.09 (bs, 1H). EIMS [$M^{+\cdot}$] m/z for $C_{19}H_{12}N_3BrS_2$ is 426.

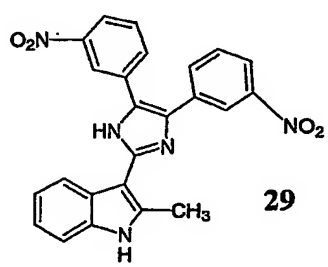
Example 9: Compound 28



28

^{1}H-NMR (DMSO-d$_6$): δ = 12.60 (s, 1H), 11.65 (s, 1H), 8.44- 8.64 (m, 3H), 8.01- 8.14 (m, 1H), 7.22-7.66 (m, 8H). EIMS [$M^{+\cdot}$] m/z for $C_{22}H_{14}N_4BrF$ is 433. M.p.= decomposed at 343.

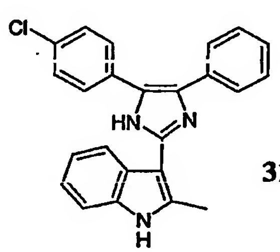
Example 10: Compound 29



29

^{1}H-NMR (DMSO-d$_6$): δ = 8.83 (q, 2H), 8.73 (m, 1H), 8.68 (d, 1H), 8.46 (d, 1H), 8.24 (s, 1H), 7.74 (t, 2H), 7.62 (t, 2H), 7.51 - 7.56 (m, 1H), 7.23 - 7.27 (m, 2H), 2.71 (s, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{23}H_{15}N_3$ is 303. M.p.= 135-137.

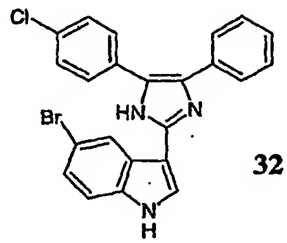
Example 11: Compound 31



31

^{1}H-NMR ($CDCl_3$): δ = 8.90 (bs, 1H), 7.62 (bs, 1H), 7.48 (bd, 4H), 7.34 (m, 4H), 7.21 (m, 1H), 7.13 (m, 2H), 2.43 (bs, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{23}H_{15}N_3ClBr$ is 448. M.p.= decomposed at 218-220.

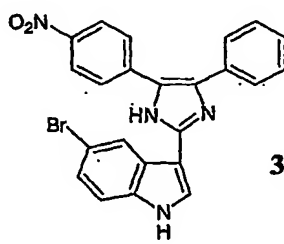
Example 12: Compound 32



32

^{1}H-NMR ($CDCl_3$): δ = 8.12 (bs, 1H), 7.48 (d, 2H), 7.46 (d, 2H), 7.23-7.34 (m, 8H). M.p.=230-232.

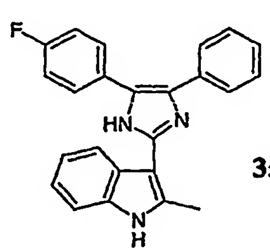
Example 13: Compound 34



34

^{1}H-NMR (DMSO-d_6): δ = 12.63 (s, 1H), 11.67 (s, 1H), 8.62 (d, 1H), 8.21 (d, 2H), 8.08 (d, 1H), 7.86 (d, 2H), 7.39-7.64 (m, 6H), 7.32 (dd, 1H). EIMS [$M^{+\cdot}$] m/z for $C_{23}H_{15}N_4BrFO_2$ is 459. M.p.= decomposed at 250-253.

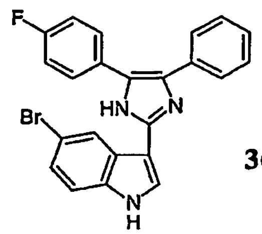
Example 14: Compound 35



35

^{1}H-NMR ($CDCl_3$): δ = 7.78 (bs, 1H), 7.59 (d, 2H), 7.54 (d, 2H), 7.35 - 7.39 (m, 2H), 7.28 - 7.34 (m,2H), 7.13 - 7.18 (m, 2H), 7.01 - 7.05 (m, 2H), 2.72 (bs, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{24}H_{18}N_3F$ is 367. M.p.= decomposed at 247-250.

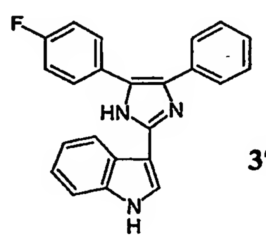
Example 15: Compound 36



36

^{1}H-NMR ($CDCl_3$): δ = 10.42 (bs,1H), 7.86 (s, 1H), 7.16-7.33 (m, 6H), 7.04 (dd, 2H), 6.95 (dd, 2H), 6.88 (t, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{23}H_{15}N_3BrF$ is 432. M.p.= decomposed at 83-86.

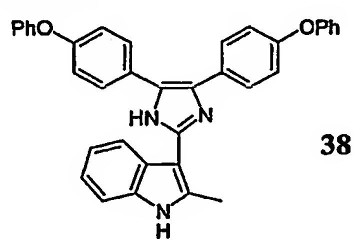
Example 16: Compound 37



37

^{1}H-NMR ($CDCl_3$): δ = 9.92 (bs, 1H), 8.17 (bs, 1H), 7.87 (t, 1H), 7.55 (bs, 1H), 7.21-7.33 (m, 6H), 7.15-7.2 (m, 1H), 7.04-7.07 (m, 2H), 6.90 (t, 2H)). EIMS [$M^{+\cdot}$] m/z for $C_{23}H_{16}N_3F$ is 353. M.p.= 51.

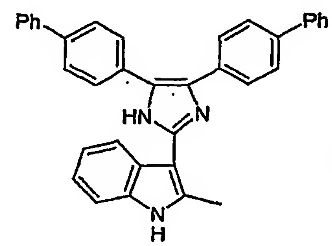
Example 17: Compound 38



38

^{1}H-NMR (Acetone-d_6): δ = 11.12 (bs, 1H), 10.46 (bs, 1H), 8.12 (d, 1H), 7.80 (bd, 2H), 7.62 (bd, 2H), 7.38-7.48 (m, 5H), 6.98 - 7.22 (m, 12H), 2.84 (bs, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{36}H_{27}N_3O_2$ is 533. M.p.= decomposed at 128-130.

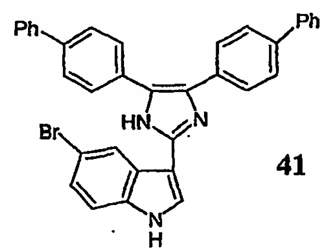
Example 18: Compound 40



40

^{1}H-NMR ($CDCl_3$): δ = 8.12 (dd, 2H), 7.60 (m, 6H), 7.24-7.53 (m, 10H), 6.87 (bd, 2H), 6.61 (bd, 2H), 2.08 (s, 3H). M.p.= decomposed at 142.

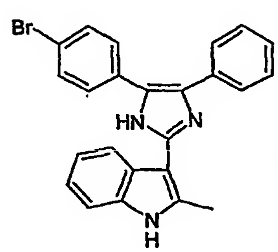
Example 19: Compound 41



41

^{1}H-NMR ($CDCl_3$): δ = 8.08 (d, 4H), 8.07 (bs, 1H), 7.75 (d, 4H), 7.28-7.50 (m, 10H), 7.12 (bd, 2H), 6.97 (bs, 1H). M.p.= 155-158.

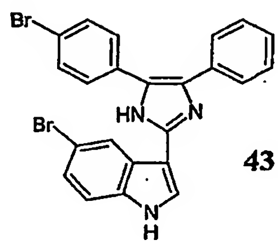
Example 20: Compound 42



42

^{1}H-NMR ($CDCl_3$): δ = 9.39 (bs,1H), 7.39 - 7.50 (m, 4H), 7.28 - 7.38 (m, 6H), 7.06 (bs, 1H), 6.94 (bs, 2H), 2.08 (bs, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{24}H_{18}N_3Br$ is 428. M.p.= decomposed at 155-157.

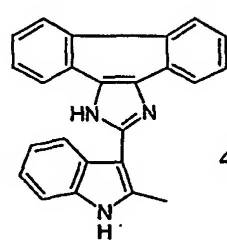
Example 21: Compound 43



43

^{1}H-NMR ($CDCl_3$): δ = 9.75 (bs, 1H), 7.83 (bs, 1H), 7.36 (m, 3H), 7.25 - 7.29 (m, 5H), 7.12 (m, 3H), 7.10 (bd, 1H). EIMS [$M^{+\cdot}$] m/z for $C_{23}H_{15}N_3Br_2$ is 493. M.p.= decomposed at 230.

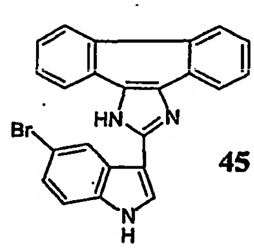
Example 22: Compound 44



44

^{1}H-NMR ($CDCl_3$): δ = 8.78 (dd, 2H), 8.19 (dd, 1H), 7.96 (bs, 1H), 7.80 (dd, 1H), 7.80 (dd, 1H), 7.55-7.77 (m, 6H), 7.16-7.42 (m, 2H), 2.87 (bs, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{24}H_{17}N_3$ is 347. M.p.= decomposed at 167.

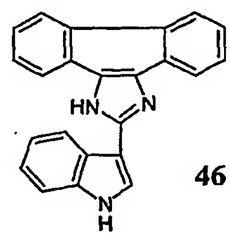
Example 23: Compound 45



45

^{1}H-NMR (DMSO-d_6): δ = 13.30 (bs, 1H), 11.62 (d, 1H), 8.87 (bd, 2H), 8.64 (bs, 1H), 8.44 (bs, 1H), 8.29 (t, 1H), 7.76 (t, 2H), 7.62 (t, 2H), 7.52 (d, 1H), 7.35 - 7.41 (m, 2H). EIMS [$M^{+\cdot}$] m/z for $C_{23}H_{14}N_3Br$ is 412.

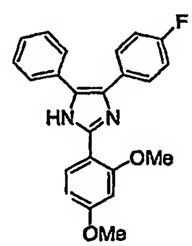
Example 24: Compound 46



46

^{1}H-NMR (DMSO-d_6): δ = 13.09 (s, 1H), 11.61 (d, 1H), 8.83 (q, 2H), 8.73 (m, 1H), 8.68 (d, 1H), 8.46 (d, 1H), 8.24 (s, 1H), 7.74 (t, 2H), 7.62 (t, 2H), 7.51 - 7.56 (m, 1H), 7.23 - 7.27 (m, 2H). EIMS [$M^{+\cdot}$] m/z for $C_{23}H_{15}N_3$ is 333. M.p.= 135-137.

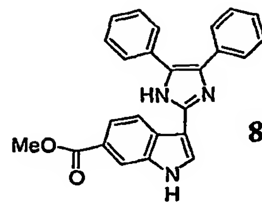
Example 25: Compound 74



74

^{1}H-NMR (DMSO-d_6): δ = 11.74 (d, 1H), 7.95 (dd, 1H), 7.32-7.57 (m, 6H), 7.17-7.31 (m, 2H), 7.12 (t, 1H), 6.70 (d, 1H), 6.66 (bd, 1H). EIMS [$M^{+\cdot}$] m/z for $C_{23}H_{19}N_2FO_2$ is 374.

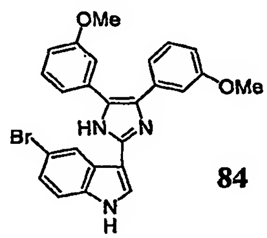
Example 26: Compound 83



83

^{1}H-NMR (DMSO-d$_6$): δ = 12.40 (s, 1H), 11.80 (s, 1H), 8.56 (d, 1H), 8.24 (s, 1H), 8.15 (s, 1H), 7.78 (d, 1H), 7.65 (d, 2H), 7.54 (d, 2H), 7.47 (t, 2H), 7.32 - 7.42 (m, 3H), 7.24 (t, 1H), 3.90 (s, 3H). EIMS [$M^{+\cdot}$] m/z for $C_{25}H_{19}N_3O_2$ is 393. M.p.= 293-295.

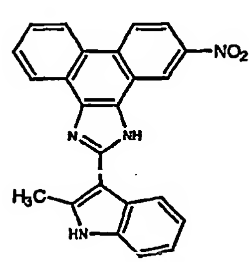
Example 27: Compound 84



84

^{1}H-NMR (DMSO-*d$_6$*): δ = 8.64 (d, 1H), 8.17 (d, 1H), 7.47 (d, 1H), 7.39 (t, 1H), 7.33 (dd, 1H), 7.20-7.31 (m, 2H), 7.12 (bd, 2H), 6.97 (bd, 1H), 6.84 (bd, 1H). 3.77 (s, 3H), 3.72 (s, 3H), EIMS [$M^{+\cdot}$] m/z for $C_{25}H_{20}N_3BrO_2$ is 474. M.p.= decomposed at 250-253.

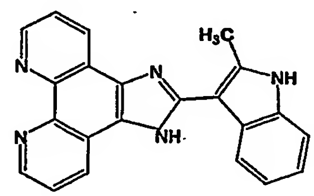
Example 28: Compound 88



Mp 335-336 ^{0}C. ^{1}H-NMR (DMSO-d$_6$), two isomers: 1) *δ* =13.160 (s) 1H, 11.602 (s) 1H, 9.720 (s) 1H, 9.143 (dd) 1H, 8.975 (dd) 1H, 8.680 (d) 1H, 8.345 (t) 1H, 8.160 (d) 1H, 7.870 (t) 1H, 7.720 (t) 1H, 7.420 (d) 1H, 7.200 (d) 2H, 2.862 (s) 3H.

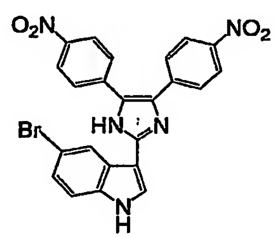
2): δ =13.090 (s) 1H, 11.602 (s) 1H, 9.370 (s) 1H, 9.143 (dd) 1H, 8.975 (dd) 1H, 8.680 (d) 1H, 8.345 (t) 1H, 8.099 (d) 1H, 7.870 (t) 1H, 7.720 (t) 1H, 7.420 (d) 1H, 7.200 (d) 2H, 2.847 (s) 3H. EI-MS ($C_{24}H_{16}N_4O_2$) = 392.

Example 29: Compound 90



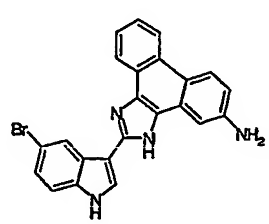
^{1}H-NMR (DMSO-d_6): δ =13.083 (s) 1H, 11.595 (s) 1H, 9.040-9.010 (m) 4H, 8.950 (d) 1H, 8.120 (m) 1H, 7.821 (t) 1H, 7.432 (m) 1H, 7.176 (m) 2H, 2.830 (s) 3H.

Example 30: Compound 92



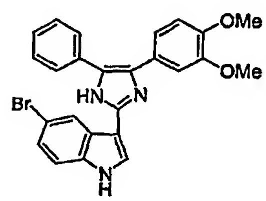
^{1}H-NMR (DMSO-d_6): δ =12.729 (s) 1H, 11.724 (s) 1H, 8.578 (d) 1H, 8.325 (d) 2H, 8.260 (d) 2H, 8.127 (d) 1H, 7.871 (m) 2H, 7.810-7.785 (m) 2H, 7.454 (d) 1H, 7.330 (d) 1H.

Example 31: Compound 94



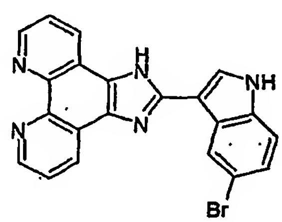
Mp 300-303 ^{0}C. ^{1}H-NMR (DMSO-d_6): δ = 12.55 (s) 1H, 8.83 (m) 3H, 8.68 (m) 1H, 8.50 (m) 1H, 8.15 (m) 1H, 7.75 (m) 2H, 7.65 (m) 1H, 7.47 (m) 1H, 7.40 (m) 1H. ESI-MS ($C_{23}H_{15}BrN_4$) = 427.

Example 32: Compound 96



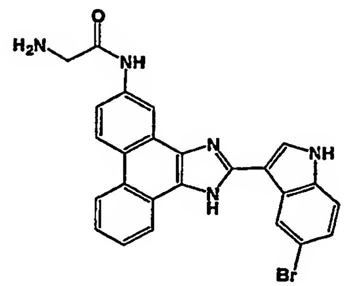
Mp 265-266 ^{0}C ^{1}H-NMR (DMSO-d_6): δ = 12.1 (s) 1H, 11.6 (s) 1H, 8.7 (d) 1H, 8.0 (d) 1H, 7.3 (m) 10H, 3.8 (d) 3H, 3.6 (d) 3H. ESI-MS ($C_{25}H_{20}BrN_3O_2$) = 474.

Example 33: Compound 97



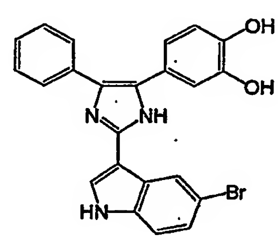
^{1}H-NMR (DMSO-d_6): δ =13.460 (s) 1H, 11.890 (s) 1H, 9.080-8.985 (m) 4H, 8.860-8.825 (m) 1H, 8.285 (d) 1H, 7.890-7.840 (m) 2H, 7.560-7.540 (m) 1H, 7.420-7.390 (m) 1H. ESI-MS ($C_{21}H_{12}BrN_5$) = 414.

Example 34: Compound 101



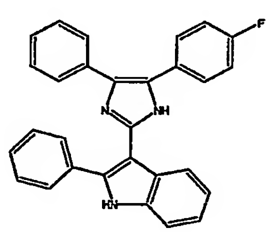
EI-MS : 484.00 ($C_{25}H_{18}BrN_5O$ requires 483.07) H^1 NMR (DMSO-d_6) d= 13.167 (s) 1H, 11.845 (s) 1H, 8.839-8.781 (m) 4H, 8.292-8.405 (m) 2H, 7.364-7.697 (m) 5H, 3.543 (s) 1H, 3.410 (s) 2H, 1.463 (s) 2H.

Example 35: Compound 140



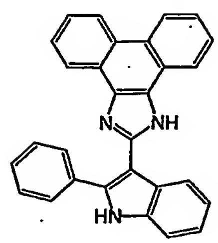
Mp 210-215 ^{0}C ^{1}H-NMR (DMSO-d_6): δ = 12.18 (s) 1H, 11.54 (s) 1H, 9.08 (d) 1H, 8.62 (s) 1H, 8.65 (s) 1H, 7.20 (m) 10H.

Example 36: Compound 141



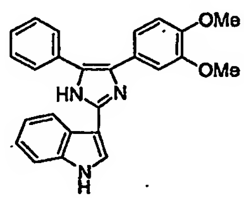
^{1}H-NMR (DMSO-d_6): δ = 12.30 (s) 1H, 11.70 (s) 1H, 7.79 (m) 3H, 7.58 (m) 2H, 7.43 (m) 7H, 7.32 (m) 2H, 7.23 (m) 2H, 7.14 (m) 2H.

Example 37: Compound 146



Mp 240-242 ^{0}C. ^{1}H-NMR (DMSO-d_6): δ = 12.20 (s) 1H, 11.90 (s) 1H, 8.85 (m) 2H, 8.60 (d) 1H, 8.40 (d) 1H, 7.70 (m) 8H, 7.30 (m) 5H.

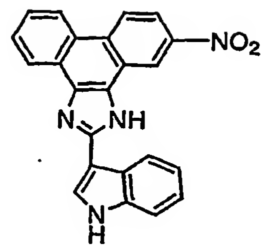
Example 38: Compound 152



Mp 258-259 ^{0}C. ^{1}H-NMR (DMSO-d_6), two isomers: 1) δ = 12.160 (s) 1H, 11.350 (s) 1H, 8.480 (t) 1H, 7.995 (d) 1H, 6.995 (d) 1H, 7.440-7.420 (m) 2H, 7.360-7.300 (m) 2H, 7.220-7.020 (m) 5H, 3.805 (s) 3H, 3.695 (s) 3H.

2) δ = 12.190 (s) 1H, 11.350 (s) 1H, 8.480 (t) 1H, 7.995 (d) 1H, 6.995 (d) 1H, 7.440-7.420 (m) 2H, 7.360-7.300 (m) 2H, 7.220-7,020 (m) 5H, 3.762 (s) 3H, 3.617 (s) 3H.

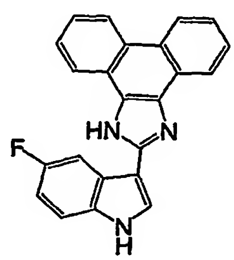
Example 39: Compound 156



Mp 365-366 ^{0}C ^{1}H-NMR (DMSO-d_6), two isomers: a) δ = 13. 410 (s) 1H, 11.670 (s) 1H, 9.40 (d) 1H, 9.11 (d) 1H, 8.96 (d) 1H, 8.70 (d) 1H, 8.35-8.18 (m) 2H, 7.96 (s) 1H, 7.70 (t) 1H, 7.56 (t) 1H, 7.28 (m) 2H.

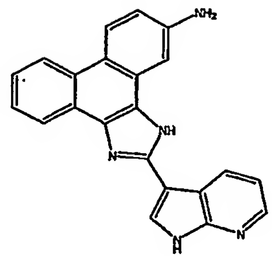
b) δ = 13.290 (s) 1H, 11.67 (s) 1H, 9.305(d) 1H, 9.095 (d) 1H, 8.960 (d) 1H, (d) 1H, 8.70 (d) 1H, 8.495 (d) 1H, 7.87 (d) 1H, 7.70 (t) 1H, 7.56 (t) 1H, 7.28 (m) 2H. EI-MS ($C_{23}H_{14}N_4O_2$) = 378.

Example 40: Compound 157



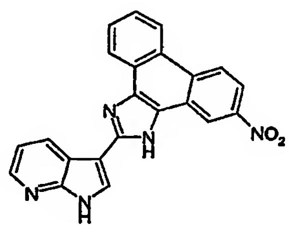
^{1}H-NMR (DMSO-d_6): δ =13.102 (s) 1H, 11.702 (s) 1H, 8.900-8.840 (m) 3H, 8.690 (d) 1H, 8.445-8.400 (m) 3H, 8.301 (d) 1H, 7.747 (t) 1H, 7.644-7.624 (m) 1H, 7.622-7.605 (m) 1H, 7.585-7.529 (m) 1H, 7.128-7.086 (m) 1H. EI-MS ($C_{23}H_{14}N_3F$) = 351.

Example 41: Compound 160



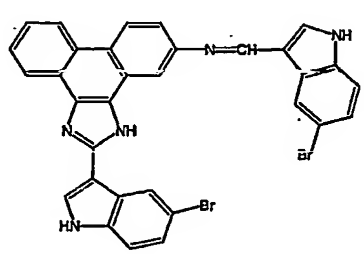
Mp 320=330 ^{0}C. ^{1}H-NMR (DMSO-d_6): δ = 13.30 (exc.) 1H, 12.92 (d) 1H, 8.64 (d) 1H, 8.56 (d) 1H, 8.43 (m) 2H, 7.62 (m) 4H, 7.34 (t) 1H, 7.03 (d) 1H, 5.95 (exc.) 2H.

Example 42: Compound 162



Mp >400 ^{0}C. ^{1}H NM R (DMSO-d_6): δ = 13. (s) 1H, 12.25 (s) 1H, 9.41 (d) 1H, 9.13 (t) 1H, 8.98 (m) 2H, 8.37 (m) 3H, 7.96 (s) 1H, 7.89 (t) 1H, 7.73 (t) 1H, 7.36 (m) 1H. EI-MS ($C_{22}H_{13}N_5O_2$) = 379.

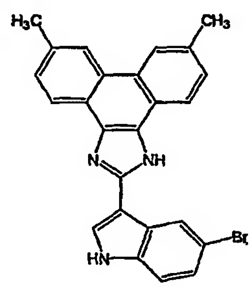
Example 43: Compound 169



169

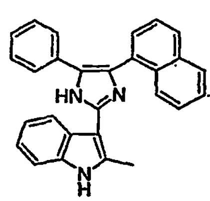
Mp 236-237 ^{0}C. EI-MS: 633.87 ($C_{32}H_{19}Br_2N_5$) require 633.33. ^{1}H-NMR (DMSO-d_6), two isomers: 1) δ =13.190 (s) 1H, 11820 (s) 1H, 8.955 (s) 1H, 8.910-8.883 (m) 3H, 8.640 (d) 1H, 8.590 (d) 1H, 8.280 (d) 2H, 8.157 (d) 1H 7.730 (t) 1H, 8.620 (t) 1H, 7.525 (s) 1H, 7.509 (s) 1H, 7.410 (d) 1H, 7.375 (d) 1H.
2) δ =13.190 (s) 1H, 12.060 (s) 1H, 8.955 (s) 1H, 8.910-8.883 (m) 3H, 8.640 (d) 1H, 8.590 (d) 1H, 8.280 (d) 2H, 8.157 (d) 1H 7.730 (t) 1H, 8.620 (t) 1H, 7.525 (s) 1H, 7.509 (s) 1H, 7.410 (d) 1H, 7.375 (d) 1H.

Example 44: Compound 175



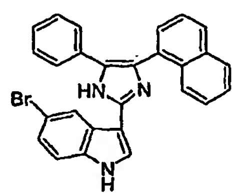
EI-MS: 440.11 ($C_{25}H_{18}BrN_3$ requires 440.07. ^{1}H-NMR (DMSO-d_6): δ =13.141 (s) 1H, 11.779 (s) 1H, 8.842 (d) 1H, 8.673 (s) 1H, 8.636 (s) 1H, 8.508 (d) 1H, 8.295 (d) 1H, 8.242 (d) 1H, 7.553 (d) 2H, 7.508 (d) 1H, 7.365 (d) 1H, 2.612 (s) 6H.

Example 45: Compound 180



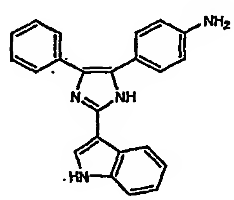
Mp 239-240 ^{0}C. ^{1}H-NMR (DMSO-d_6): δ = 12.12 (s) 1H, 11.30 (s) 1H, 8.07 (d) 1H, 8.03 (d) 1H, 7.93 (d) 1H, 7.72 (d) 1H, 7.65 (m) 2H, 7.54 (t) 1H, 7.47 (m) 3H, 7.28 (m) 3H, 7.08 (m) 4H, 2.70 (s) 3H. EI-MS ($C_{28}H_{21}N_3$) = 399.

Example 46: Compound 181



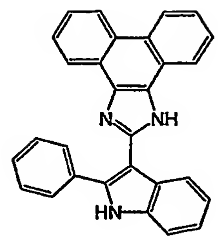
Mp 308-310 ^{0}C. ^{1}H-NMR (DMSO-d_6), two isomers: 1) δ =12.547 (s) 1H, 11.570 (s) 1H, 8.739 (d) 1H, 8.200-8.790 (m) 3H, 7.695 (m) 2H, 7.570-7.060 (m) 10H.
2) δ =12.575 (s) 1H, 11.620 (s) 1H, 8.478 (d) 1H, 8.200-8.790 (m) 3H, 7.695 (m) 2H, 7.570-7.060 (m) 10H. ESI-MS ($C_{27}H_{18}BrN_3$) = 464.

Example 47: Compound 182



Mp 264-265 ^{0}C. ^{1}H-NMR (DMSO-d_6): δ = 12.0 (s) 1H, 11.3 (s) 1H, 8.46 (d) 1H, 7.96 (d) 1H, 7.67 (d) 2H, 7.51 (d) 1H, 7.43 (d) 1H, 7.39 (t) 1H, 7.28 (t) 2H, 7.15 (m) 1H, 6.62 (d) 2H. EI-MS ($C_{23}H_{18}N_4$) = 350.

Example 48: Compound 183



Mp 240-242 ^{0}C. ^{1}H-NMR (DMSO-d_6): δ = 12.20 (s) 1H, 11.90 (s) 1H, 8.85 (m) 2H, 8.60 (d) 1H, 8.40 (d) 1H, 7.70 (m) 8H, 7.30 (m) 5H.

Example 49: *In vitro* Inhibition of Proliferation of Cancer Cells #1

Selected compounds of Formula I were tested for anti-cancer activity *in vitro* using a human colon carcinoma cells (HT-29) and human non-small cell lung cancer cells (H460). The cells were maintained in α-MEM medium (Wisent, St-Bruno, Qc) supplemented with 10% FBS, and grown at 37°C in an atmosphere of 5% CO_2. Cells were transferred onto 150mm tissue culture plates and grown until sub-confluency (70-80%) prior to their use.

The anti-cancer activity *in vitro* was evaluated by a cell proliferation assay based on the ability of live cells to reduce the tetrazolium salt XTT to orange coloured compounds of formazan (XTT cell proliferation kit II, Roche Applied Science, Montreal, QC).

Approximately 4 x 10^3 colon cancer cells (HT-29) or 2 x 10^3 non-small cell lung cancer cells (NCI-H460) in 100 μl of complete culture medium were plated onto 96-well microtiter plates and incubated overnight at 37°C. The medium was then removed by inverting the plate and patting on a sterile absorbent cloth. Fifty μl of medium containing the test compound at either 25 or 100 μM, were added to the wells containing cells and incubated at 37°C in an atmosphere of 5% CO_2 for 48 h. Following incubation, 25 μl of an XTT reaction mixture (XTT at a final concentration of 0.3 mg/ml) were added to each well and the plates were incubated for a further 4 h. The absorbance of each sample was then determined at a wavelength of 490 nm/650 nm as reference. Each compound was tested in duplicate and the results are reported

as averages. Table II shows the effect that different compounds of Formula I have on the growth of human colon carcinoma HT-29. Table III shows the effect that different compounds of Formula I have on the growth of human non-small cell lung cancer cells (H460).

Table II: Inhibition of Proliferation of Human Colon Carcinoma (HT-29) Cells

Compound	100μM		25μM	
	% Survival	SD(%)	% Survival	SD(%)
5	110.7	1.9	110.9	2.8
6	3.2	0.2	11.7	1.6
9	15.1	2.8	68.3	16
10	7.6	0.5	25.8	2.6
11	94.3	3.6	107.8	1
13	82.4	0.8	105.9	5.4
14	3.8	0.5	55.2	15.7
19	37.1	7.2	105.5	2.9
20	28.1	5	100	2.7
23	45.7	5.8	98.2	0
25	39.8	4.7	63.9	1.6
27	35	0.6	62.3	2.4
29	20.9	1	37.1	6.2
31	24.9	1.8	98.6	3.3
32	7.7	0.6	22.7	0
33	10	0.3	56.1	5.9
34	10.8	0	22.8	2
35	2.5	0.3	44.1	4.4
36	4.7	0.8	31.6	2.2
38	35.7	1.5	67	7.9
39	53	0.3	96.2	4
40	36.7	1.8	79.1	1.4
42	1.8	0	59.9	0.1
43	5.7	0.3	28	6.8

Compound	100μM		25μM	
	% Survival	SD(%)	% Survival	SD(%)
44	6.5	0.4	63.6	2.9
45	35	0.6	88.9	3.3
46	4.5	0	16.1	1
73	62.2	3.2	65	2
83	109.5	4.7	100.3	1.1
CPT-11	51.1	3.2	82.3	10
Vehicle	100	7	100	7

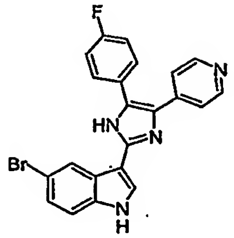
Table III: Inhibition of Proliferation of Human Lung Carcinoma (NCI- 460) Cells

Compound	100μM		25μM	
	% Survival	SD(%)	% Survival	SD(%)
5	106	2.6	102	0.7
6	1.9	0.5	10.4	0.8
9	8.4	1.6	98.2	1
10	2.7	0.1	26.9	1.6
11	101.6	8.3	98.8	3.3
13	96.2	1.1	101.9	4
14	1.8	0.1	83.5	20.4
19	27.3	6.1	89.2	0.2
20	82.1	20.6	98.6	2.1
23	92.1	0	96.3	0.9
25	89	3.4	99.5	0.8
27	43.1	1.4	93.5	0.2
29	20.2	1.4	73.8	2.2
31	37.6	5.6	94	2.3
32	2.9	0.5	15	0.2
33	9.4	2	73.4	2.2

Compound	100µM		25µM	
	% Survival	SD(%)	% Survival	SD(%)
34	7.6	0.9	17.4	0.2
35	1.2	0.1	83.8	8
36	2.4	0.3	24.5	2.1
38	12.2	1.6	98.8	2
39	17	0.7	98.1	0.3
40	7.7	0.5	97.6	3
42	1.2	0.1	66.1	16.9
43	3	0.1	18.8	1.5
44	3.4	1	77.3	5.3
45	32.7	5.5	96	1.1
46	1.9	0.1	11.5	1.4
73	53.5	1.3	89.2	0.6
83	109.2	1.5	100.9	2.6
CPT-11	6.1	0.5	32.2	4.5
Vehicle	100	3.4	100	3.4

Example 50: *In vitro* Inhibition of Proliferation of Cancer Cells #2

The compounds listed below were tested for anti-cancer activity against several carcinoma cell lines as described below and in Examples 51-53.



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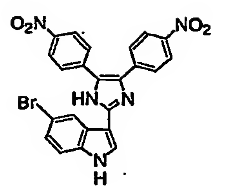

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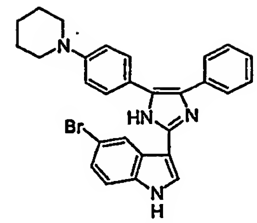

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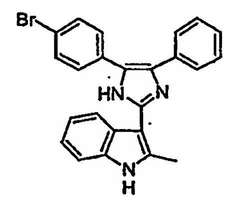

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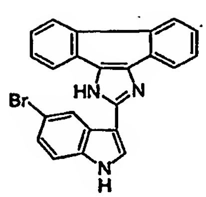

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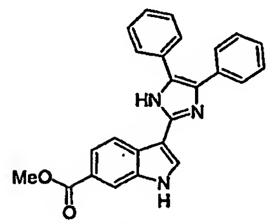

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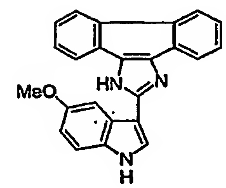

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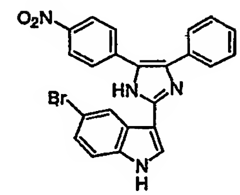

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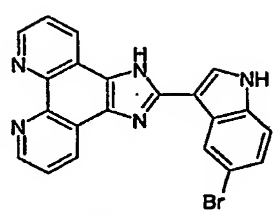

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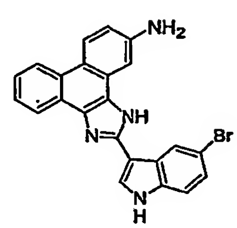

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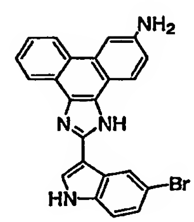

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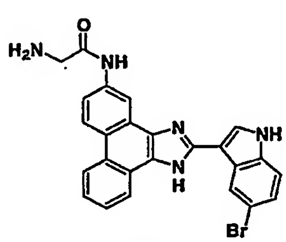

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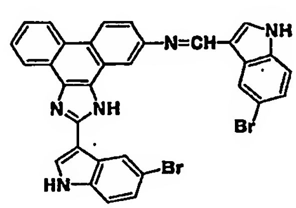

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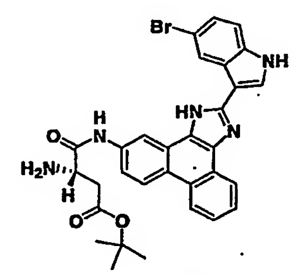

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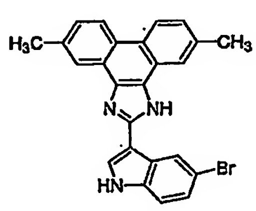

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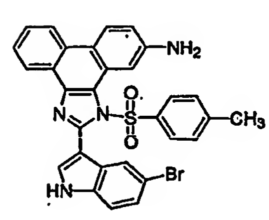

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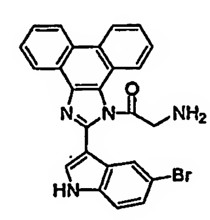

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Cells were maintained in α-MEM medium (Wisent, St-Bruno, QC) supplemented with 10% FBS, and grown at 37°C in an atmosphere of 5% CO_2. They were transferred onto 150mm tissue culture plates and grown until sub-confluency (70-80%) prior to their use.

The anti-cancer activity *in vitro* was evaluated by a cell proliferation assay based in the ability of live cells to reduce the tetrazolium salt XTT to orange coloured compounds of formazan (XTT cell proliferation kit II, Roche Applied Science, Montreal, QC). The following cancer cell lines were tested: HT-29 colon carcinoma, A498 renal carcinoma, Caki-1 renal carcinoma, C8161 melanoma, MDA-MB-231 breast adenocarcinoma, A2058 metastatic melanoma, SK-OV-3 ovarian adenocarcinoma, Hep G2 liver carcinoma, AsPC-1 pancreatic adenocarcinoma, PC3 metastatic prostate adenocarcinoma. WI 38 is a human lung fibroblast cell line.

Approximately 2-3×10^3 cells in 100 μl of complete culture medium were plated onto 96-well microtiter plates and incubated overnight at 37°C, the medium was removed by inverting plate and patting on sterile absorbent cloth. Fifty μl of medium containing the different compounds at different concentrations were added and wells were incubated at 37°C with 5% CO_2 for 48 h. Following incubation, 25 μl of an XTT reaction mixture (XTT at a final concentration of 0.3 mg/ml) were added and wells were incubated for 4 h. The absorbance of each sample was determined at a wavelength of 490nm/650 nm as reference. The percentage of survival was determined by the ratio between absorbance values of cells incubated with the different compounds and their respective controls (cells incubated with vehicle only). The results are shown in Figures 1-4.

Figure 1 depicts the results with compound **92**; Figure 2 depicts the results with compound **28**; Figure 3 depicts the results with compound **50**; and Figure 4 depicts the results with compound **42**.

Example 51: Concentration Dependence of Inhibition of Cancer Cell Proliferation by Compound 45 *in vitro*

The effect of various concentrations of compound **45** on various cancer cell lines was tested following the general protocol outlined in Example 50, with the following exceptions. Cell survival was assessed 48 h, 72 h and 6 days post-treatment by incubating cells with XTT for 2 h. The cancer cell lines utilised in this example were the same as those listed in Example 50, together with the cervical carcinoma cell line KB. The results are shown in Figures 5 and 6, which depict cell survival after treatment with various concentrations of compound **45**. **A.** 48 h after treatment, **B.** 72 h after treatment and **C.** 6 days after treatment.

Example 52: Concentration Dependence of Inhibition of Cancer Cell Proliferation by Compounds 45, 33 and 99 *In Vitro*

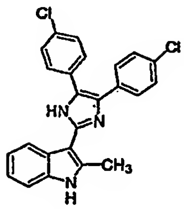
The effect of various concentrations of compounds **45**, **33** and **99** on the colon carcinoma cancer cell line LS513 was tested following the general protocol outlined in Example 50, with cell survival being assessed 6 days post-treatment. The results are shown in Figure 7.

Example 53: *In Vitro* Inhibition of Proliferation of Colon Carcinoma Cells #1

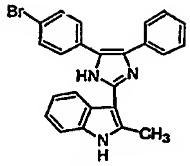
The effect of various compounds of Formula I on the proliferation of HT-29 colon carconoma cells was tested following the general protocol outlined in Example 50 with the exception that cell survival was assessed after 5 to 7 days of treatment. The results using concentrations of 2, 10 and 25μM of each compound are shown in Figure 8. Results were compiled from different experiments with 5 to 7 days of treatment. The co-efficient of variation for most samples were within 5%.

Example 54: *In Vitro* Inhibition of Proliferation of Cancer Cells #3

The twenty-three compounds of Formula I shown below were evaluated for their antiproliferative effects in a panel of 60 human cancer cell lines as part of the *in vitro* anticancer screening services provided by the DTP (Developmental Therapeutics Program) of the US National Cancer Institute (NCI) U.S. National Cancer Institute (NCI) of the National Institutes of Health (NIH) in Rockwell, Maryland. The cancer cell lines used in this screen are provided in Figure 9.



19



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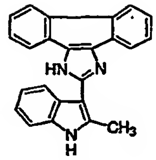

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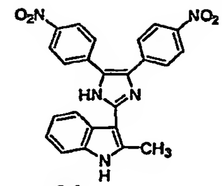

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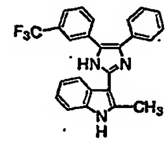

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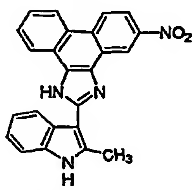

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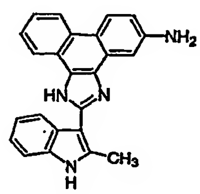

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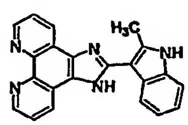

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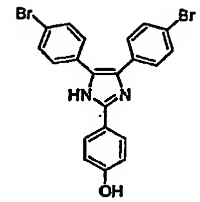

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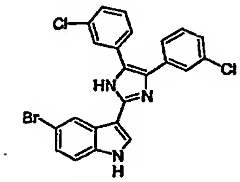

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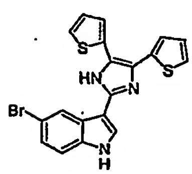

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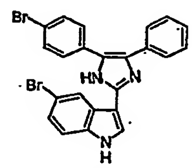

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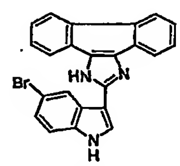

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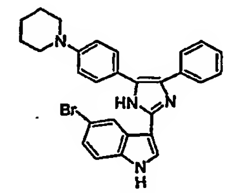

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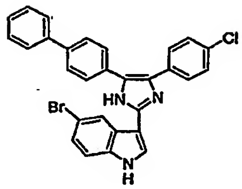

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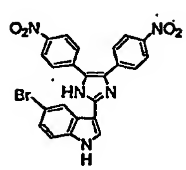

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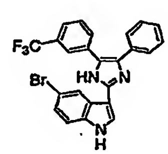

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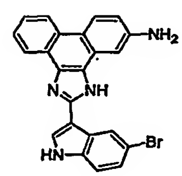

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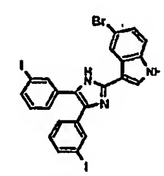

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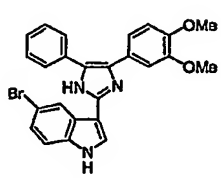

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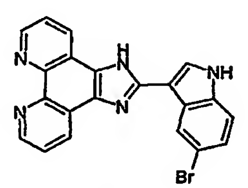

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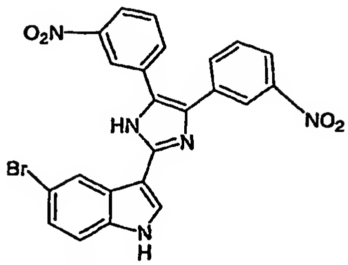
The NCI conducts a standard 48/72 hour 60 cell line assay and an *in vitro* time course assay as described in Alley *et al.* (*Cancer Res* (1988) 48:589). In the standard 60 cell line assay, a minimum of 5 concentrations of the test compound are tested at 10-fold dilutions against 60 cell lines and cell growth is assayed at 48 and 72 hours using a sulphorhodamine B assay. For the time course analysis, tumour cells are treated with

the test compound at various time points, then washed and grown in medium free of the test compound until the end of the experiment at 144 hrs. This assay employs 20% FBS to better approximate the minimum c x t (concentrations and times) test compound exposure conditions that are required to achieve activity *in vivo*. Cell growth is quantified by an MTT assay (similar to the XTT assay described above) and the concentration of the test compound required for growth inhibition is determined. The inhibitory effect of the test compounds are expressed as a GI_{50} value, which represents the molar concentration of the test compound that results in 50% growth inhibition.

All compounds exhibited antiproliferative activity against all human tumour cell lines including NSCLC, leukemia, colon cancer, prostate cancer, melanoma, ovarian cancer, renal cancer, CNS cancer, and breast cancer, with GI_{50} (growth inhibition by 50%) values ranging from 0.61 μM to 12.3 μM, with an average of 2 μM. The compound **45** had a GI_{50} value of 2.0 μM, while the most effective compound was **90** (Figure 10A). The compounds affected the growth of all cell lines comparatively equally. The average GI_{50} values for compound **45** ranged from 1.3 μM (renal) to 3.4 μM (leukemia) (Figure 10B). These results suggest that compound **45** affects a ubiquitous target. The TGI (total growth inhibition) for this compound towards leukemia cell lines was significantly different from that of other cell types. These cell lines were not 100% growth inhibited, even at 100 μM, the highest concentration used (Figure 10C).

Example 55: *In Vitro* Inhibition of Proliferation of Lung Cancer Cells

The following compounds were tested for theier ability to inhibit the proliferation of H460 non-small cell lung carcinoma cells *in vitro*. The protocol described in Example 50 was utilised with the exception that cell survival was assessed after 6 days of treatment. Each compound was tested at concentrations of 0.2, 2, 10 and 25 μM. The results are shown in Figure 11.



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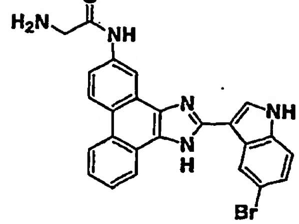

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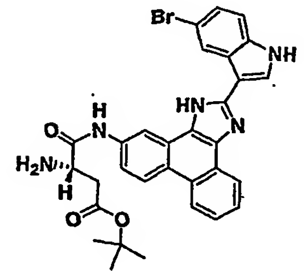

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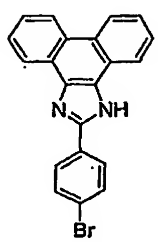

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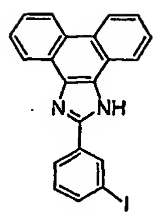

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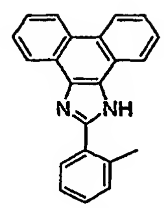

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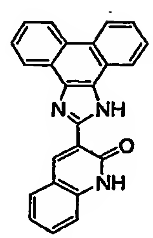

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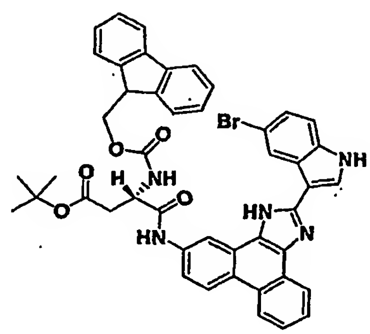

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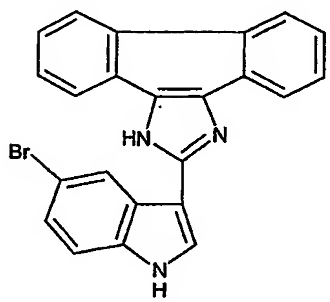
Example 56: *In Vitro* Inhibition of Proliferation of Colon Carcinoma Cells #2

The above compounds (as shown in Example 55), together with those shown below, were tested for their ability to inhibit the proliferation of HT-29 colon carcinoma cells *in vitro*. The protocol described in Example 50 was utilised with the exception that cell survival was assessed after either 2 or 6 days of treatment. Each compound was tested at concentrations of 0.2, 2, 10 and 25 μM (compounds **110, 30, 101, 113, 103, 107, 108** and **109**) or at concentrations of 2.5, 10 and 25 μM (compounds **112, 114, 78, 111** and **45**). The results are shown in Figures 12 and 13. The results shown in

Figure 12 reflect cell survival 6 days after treatment with the listed compounds. Figure 13A shows cell survival 2 days after treatment with the listed compounds and Figure 13B shows cell survival 6 days after treatment.



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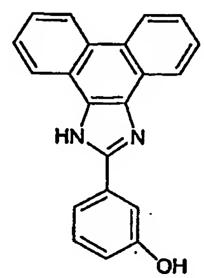

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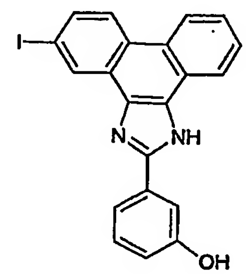

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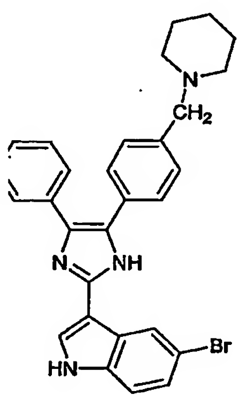

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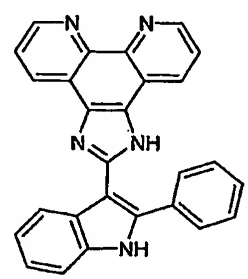

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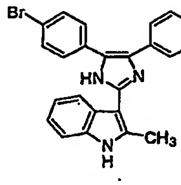
Example 57: Inhibition of Colon Carcinoma Growth *In Vivo* #1

This Example and the following Example 58 describe *in vivo* efficacy studies of various compounds of Formula I performed using a mouse xenograft model using the human colon adenocarcinoma cell line HT-29. The following compounds were tested.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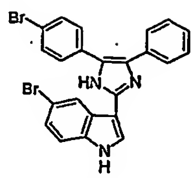
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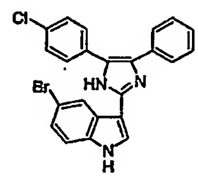
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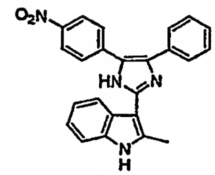
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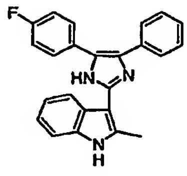
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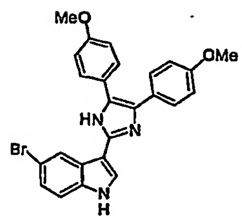
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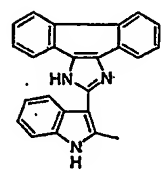
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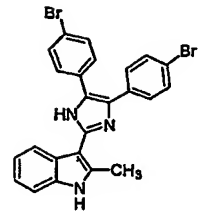
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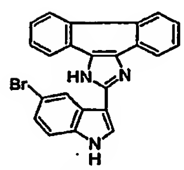
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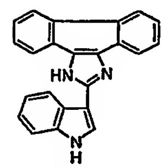
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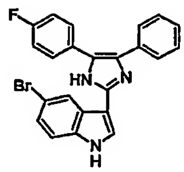
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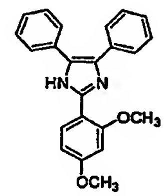
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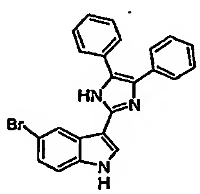
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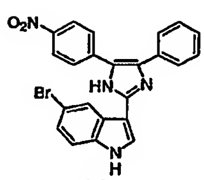
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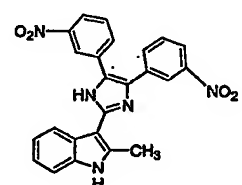
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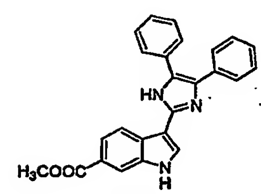
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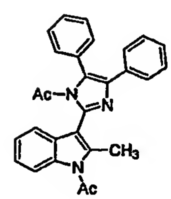
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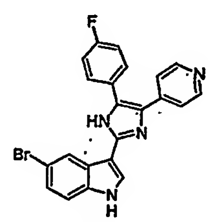
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Groups of five to 10 CD-1 female nude mice (6-7 weeks) were injected in the lower mid back with human colon adenocarcinoma cells HT-29 (3×10^6 cells in 0.1 ml PBS) subcutaneously, and the treatment initiated 5 days post-inoculation (size of tumours = 20-40 mm^3). The treatment schedule consisted of 2 x 200 μl intraperitoneal

injections per day of 5 mg/ml (100 mg/Kg/d) for five days and 2 days break, for 4 weeks. Tumour sizes were measured during the course of the treatment using calipers, mice were then sacrificed by cervical dislocation and tumours surgically removed and weighed. Figures 14 shows the average tumour size (mm^3) in the different groups of mice. Figures 15 and 16 show the average tumour weight per group of mice and per individual mouse, respectively.

Example 58: Inhibition of Colon Carcinoma Growth *In Vivo* #2

The protocol described in Example 55 was followed. The results are shown in Figure 17, which depicts the average tumour size (mm^3) in the different groups of mice. Abbreviations used in Figure 17 are as follows: V-ip = Vehicle (i.p); 42 (5-ip) = 5 mg/Kg (i.p.); 42 (25-1p)= 25 mg/Kg; 42 (100-ip); 100 mg/Kg (i.p.); 43 (ip) = 100 mg/Kg(i.p.); 45 (ip)= 100 mg/Kg (i.p); 44(ip)= 100 mg/Kg(i.p.); 46 = 100 mg/Kg (i.p); 28= 100 mg/kg (i.p); V-op = vehicle, oral; 42 (100-op) = 100 mg/Kg (oral).

Example 59: *In Vivo* Inhibition of Cancer Cell Growth by Compound 45

The ability of compound **45** to inhibit the growth of cancer cells *in vivo* was further investigated in a mouse xenograft model of hepatocellular (liver) cancer. Groups of five to 10 CD-1 female nude mice were injected subcutaneously in the mid right flank with HepG2 human hepatocarcinoma cells (1 x 10^7 cells). The treatment was initiated 7 days post-inoculation and consisted of 2 x 200 μl intraperitoneal injections per day (100 mg/Kg/d). Tumour sizes were measured during the course of the treatment using calipers, and were surgically removed and weighed after 10 weeks. The results obtained are shown in Figure 18A & B.

Notably, none of the compounds tested in the preceding Examples 57-59 showed toxic effects *in vivo*.

Example 60: Effect of Compound 45 on the Activity of Various Human Kinase Enzymes #1

Compound 45 was tested for its ability to function as a kinase inhibitor using the kinase profiler service from Upstate Biotechnologies. The general protocol employed is as follows: recombinant kinases were incubated with specific substrates, 10 mM MgAcetate, and [γ-^{33}P-ATP]. The reaction was initiated by the addition of MgATP mix. After incubation at room temperature for 40 minutes, the reaction was stopped by the addition of 5 µl of a 3% phosphoric acid solution. 10 µl of the reaction was then spotted on to a P30 filtermat and washed 3 times for 5 min. in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting. Each reaction was performed in duplicate with 100 µM ATP -/+ 10 µM compound 45. Results are presented in Table IV and are expressed as the mean of % control (no compound). PI 3-kinase-γ (PI3K-γ) activity was determined with the PIProfiler™ assay, which measures the binding of the GRP1 pleckstrin homology (PH) domain to PIP3, the product of PI3K acting on its physiological substrate PIP2.

Seventy-nine recombinant kinases were tested. Of these 87% retained greater then 60% activity in the presence of 10 µM ML-220. Four kinases retained between 40 and 60% activity (Alk-60%; Aurora-A, 54%; PKD2, 52%; SAPK3, 54%; TrkA, 56%), whereas 5 kinases had less than 40% activity (CaMKII, 32%; PI3Kα, 30%; PI3Kβ, 11%; PI3Kδ, 9%; and PI3Kγ, 22%). These results indicate that compound 45 can function as a kinase inhibitor, and it has a high degree of selectivity for particular kinases.

Table IV: Kinase Inhibiting Activity of Compound 45

Kinase	Family	% activity
Abl	TK	107
ALK	TK	60
AMPK	CAMK	100
ASK1	STE	99
Aurora-A	other	54

Kinase	Family	% activity
Axl	TK	100
BRK	TK	112
CaMKII	CAMK	32
CaMKIV	CAMK	98
CDK1/cyclinB	CMGC	156
CDK2/cyclinA	CMGC	95
CDK2/cyclinE	CMGC	117
CDK3/cyclinE	CMGC	107
CDK6/cyclinD3	CMGC	87
CDK7/cyclinH/MAT1	CMGC	95
CHK1	CAMK	111
CK2	other	91
EGFR	TK	105
EphA2	TK	95
EphB4	TK	95
ErbB4	TK	73
Fes	TK	99
FGFR3	TK	82
Fms	TK	135
Fyn	TK	103
GSK3α	CMGC	96
IGF-1R	TK	80
IKKß	other	111
IKKα	other	150
JNK1α1	CMGC	85
JNK3	CMGC	121
Lyn	TK	81
MAPK1	CMGC	85
MAPK2	CMGC	99
MAPKAP-K2	CAMK	119
MEK1	STE	88
Met	TK	129

Kinase	Family	% activity
MINK	STE	91
MKK4	STE	96
MKK6	STE	86
MSK1	AGC	76
MST2	STE	77
NEK2	other	90
p70S6K	AGC	64
PAK2	STE	89
PAR-1Bα	CAMK	88
PDGFRα	TK	117
PDK1	AGC	106
PI3K□	LIPID	22
PI3K-ß	LIPID	11
PI3K-α	LIPID	30
PI3K-δ	LIPID	9
Pim-1	CAMK	70
PKA	AGC	83
PKBα	AGC	95
PKCμ	AGC	90
PKCα	AGC	92
PKCδ	AGC	87
PKCζ	AGC	96
PKD2	CAMK	52
Plk3	other	132
PRK2	AGC	83
RAF	TKL	100
Ret	TK	82
ROCK-II	AGC	86
Ros	TK	107
Rse	TK	176
Rsk1	AGC	183
SAPK2a	CMGC	62

Kinase	Family	% activity
SAPK2b	CMGC	80
SAPK3	CMGC	54
SAPK4	CMGC	77
SGK	AGC	89
SRC	TK	102
TAK1	TKL	104
Tie2	TK	109
TrkA	TK	56
Yes	TK	91

Example 61: Effect of Other Compounds of Formula I on the Activity of Various Human Kinase Enzymes

To assess whether other compounds of Formula I also affected the same kinases, the inhibitory activity of 10 μM of compound **30** or compound **90** was tested on five kinases: Aurora-A, CaMKII, PKD2, SAPK3, TrkA and PI3K. The results are shown in Figure 19 The results indicated that these two compounds have a different pattern of kinase inhibition than compound 45.

Example 62: Determination of the Subcellular Localization of Compound 45 in Various Cancer Cells

Compound 45 is intrinsically fluorescent, which allowed the subcellular localization of this compound to be examined by fluorescent microscopy. Fluorescent microscopy was performed at the Microscopy Imaging Centre, Faculty of Medicine, University of Toronto. Cells were treated with 100 μM of compound **45** (Figure 20A, B, D, E) or 1 μM doxorubicin (Figure 20C) for 1 hour, washed once in PBS, fixed in 3.7% formaldehyde/PBS for 10 minutes, washed three times in PBS and mounted with Immuno-fluoro. Images were obtained with a Zeiss laser scanning fluorescent microscope with an excitation filter range of 360-370 nm (compound **45**) or 530-560

nm (doxorubicin). For Figure 20B and C, differential interference contrast images were overlaid with fluorescent images.

Compound **45** localizes to punctuate spots in the perinuclear area of HT-29 colon adenocarcinoma cells (Figure 20A), and is excluded from the nucleus and plasma membrane regions (Figure 20B). In contrast, the anti-cancer agent, doxorubicin, which is also intrinsically fluorescent, is localized in the nucleus (Figure 20C). A similar localization for compound **45** was observed in A498 renal carcinoma cells (Figure 20D) and C8161 melanoma cells (Figure 20E).

Example 63: Determination of Morphological Changes in Cells Treated with Compound 45

Treatment with compound **45** for 24 hours leads to the formation of large vacuoles within the cytoplasm of HT29 colon adenocarcinoma cells (Figure 21), A498 renal carcinoma cells and MDA-MB-231 breast adenocarcinoma cells. These vacuoles are not formed in DMSO- or doxorubicin-treated cells. Moreover, the nuclear membrane is no longer evident in the phase-contrast images of cells treated with compound **45**, even though the nucleus is still intact, as shown by DAPI staining. Figure 21 shows differential interference contrast (DIC) images (top row) and fluorescent images (lower row) of the same cells stained with DAPI, a cell permeable marker for the nucleus.

Example 64: Cell Cycle Analysis

The effect of treatment with compound **45** on cell cycle progression in HT-29 colon adenocarcinoma cells was examined by flow cytometry (Figure 22). Values were determined by gate analysis of flow cytometric plots and are presented in Figure 22 as a percentage of the total cell population, after eliminating doubles. Apoptotic events inferred by the surface area preceding G1 phase. Cells were starved for 3 days, and treated with 15 μM or 25 μM compound **45** for 24 or 48 hours in the presence of 10% serum, followed by flow cytometric analysis. Treatment with compound **45** led to an increase of cells in the G1 phase and a decrease in the S and G2/M phases.

The results presented above in this Example and in Examples 58, 60 and 61 indicate that compound **45** suppressed the growth of HT-29 colon cancer cells with a GI_{50} of 2.6 μM, and induced a partial arrest in the G0/G1 phase of the cell cycle. Fluorescent microscopy revealed the presence of compound **45** within the cytoplasm, but not the nucleus or plasma membrane regions of the cell. In addition, compound **45** was found to inhibit kinase activity in a screen of protein kinases, indicating that the cellular target may be a cytoplasmic protein kinase. These results indicate that compound **45** and related derivatives have potential as therapeutic agents for the treatment of human cancer.

Example 65: Selectivity of Compounds of Formula I

Compounds of Formula I that demonstrate the ability to decrease the growth or proliferation of at least one cancer cell line may undergo further testing to evaluate their selectivity towards cancer cells. An exemplary method to measure the selectivity of the compounds of the present invention is provided below.

IC_{90} values of selected compounds on a panel of normal actively proliferating cells (HUVEC and WI38) and cancer cells representing colon (HT-29), lung (NCI-H460), breast (MDA-MB-231) and prostate cancer (PC-3) are measured. Compounds with 2-fold or higher overall selectivity to the panel of cancer cell lines at IC_{90} are identified as potential therapeutics.

IC_{90} values are determined using the XTT assay as an indicator of growth arrest and/or cytotoxicity. This assay is conducted as outlined in Example 50. Percentage inhibition is calculated for each cell line and IC_{90} values for each compound and cell type determined. The average IC_{90} values for the normal cells are calculated and divided by the average IC_{90} values for the cancer cell lines. Compounds with a selectivity ratio of >2 are identified and chosen for further optimization and/or testing.

Example 66: Additional *In Vivo* Anti-tumour Efficacy Evaluations

Further pharmacological evaluation of selected compounds is conducted in animal models of human tumour growth. Data from these studies provide evidence of the

therapeutic efficacy of selected compounds against various types of cancer and help to identify compounds with better pharmacological properties and potency.

Examples of mouse models that can be utilized to investigate the efficacy of selected compounds include, but are not limited to, xenografts of various human tumour types, inoculated subcutaneously into nude mice or mice with severe combined immunodeficiency disorder (SCID) as described above; orthotopic implantation of various human tumours in nude or SCID mice for investigation of effects on the tumour in the target organ (for example, a pancreatic cancer cell graft implanted directly into the pancreas of the animal), and investigation of spontaneous tumours in normal mice.

In order to provide evidence of the efficacy of a selected compound as a single agent, it may be evaluated, for example, in specific models (xenograft or orthotopic) for representative human cancers such as pancreas, skin (melanoma), kidney, colon, breast, lung, liver, ovary, prostate, bladder and brain. Similar studies can be conducted to evaluate the performance of test compounds in combination with other standard therapeutic modalities used in the treatment of human cancers.

For typical xenograft studies, 5-6 week old, female, CD-1 athymic nude mice, (Charles River, Montreal, QC) are acclimatized in a pathogen-free facility for at least 1 week. Animal protocols followed are in compliance with the Guide for the Care and Use of Laboratory Animals in Canada. Approximately 10^6-10^7 human tumour cells in 100 ml PBS are subcutaneously injected into the right flank of each mouse. Once tumours reach an approximate volume of 100 mm^3 (several days post tumour cell injection), mice are randomized by tumour size into control and treatment groups. Test compounds are administered at various doses 5 days a week for several weeks. Control animals receive vehicle alone (negative control) and/or a standard chemotherapeutic (positive control) for the same period. The tumour dimensions (length, width, and height) are measured using calipers twice a week over the treatment period. Tumour volume is calculated by the formula L x W x H/2, where L indicates length, W indicates width and H indicates height. The mice are sacrificed when the tumour burden reaches approximately 10% of total body weight and excised

tumours are weighed. A standard bar graph is used to demonstrate the differences in tumour weights with each bar representing mean tumour weight.

Example 67: Additional Assays to Investigate Potential Mechanism of Action

The potential mechanism of action of selected compounds can be investigated using assays such as cell-cycle analysis, apoptosis assays, anti-angiogenesis assays and immunohistochemical analysis. A representative example of each type of assay is provided below.

i) Cell-cycle analysis

Alterations in cell cycle are determined using flow cytometric analyses. Tumour cells sensitive to a test compound are synchronized by plating in medium containing 0.5% FBS for 24 h followed by culturing in FBS-free medium for 48 h. The cells are then released into complete medium containing 0.1% DMSO (vehicle control) or the test compound at an appropriate concentration (*e.g.* 3 x IC_{90} value), harvested 16 to 24 h following treatment, washed twice with cold PBS and fixed in 70% ethanol at 4° C for at least 4 h. The fixed cells are centrifuged at 1500rpm for 4 minute at 4° C, washed twice with cold PBS containing 2% FBS, treated with 3 mg/ml ribonuclease (Sigma Chemical Co. Oakville, ON) and 50 μg/ml propidium iodide (PI) (Sigma Chemical Co.) for 30 minutes at 37° C. The fluorescence of the stained cells is measured using a FACScan flow cytometer and the Cell Quest program (Becton Dickinson, San Jose, CA). Data are evaluated using Modfit software (Verity software House, Topsham, ME) and the effects of the selected compounds on cell cycle are evaluated.

ii) Apoptosis assay.

DNA fragmentation analysis is used to evaluate the apoptotic effects of test compounds. Briefly, cells are plated in six-well culture plates 24 hr prior to treatment. After incubation with the test compound, medium containing detached cells is transferred to 15 ml conical tubes while cells still attached to the plate are trypsinized and then added to the same tubes. After centrifugation, collected cells are washed with PBS and resuspended in 0.5 ml lysis buffer containing 50 mM Tris-HCl, pH 8.0, 1.0 M NaCl, 10 mM EDTA and 0.5% SDS. Cell lysates are transferred to

microfuge tubes and proteinase K is added to a final concentration of 0.2 ml/ml and incubated overnight at 37° C. DNA is extracted by phenol:chloroform:isoamyl alcohol (24:24:1), dried and dissolved in 40 μM of 10 μM Tris-HCl (pH 8.0) and 0.1 mM EDTA. DNase-free RNase A is added to each sample for 30 min at 37° C and 12 μl of each sample are loaded onto a 2% agarose gel containing 0.5 μg/ml ethidium bromide and electrophoresed. DNA is visualized under UV illumination and the induction of apoptosis by the test compound is evaluated based on the generation of a nucleosomal-size DNA ladder.

iii) Anti-angiogenesis assay

Proliferation of new capillaries, *i.e.* angiogenesis or neovascularization, is critical for the transition of a small localized tumour to expand into a large malignant growth. The Matrigel Plug Assay (see, Passaniti *et al., Lab. Invest.* (1992) 67:519) is a simple method for assessing angiogenesis and the possible anti-angiogenic effect of selected compounds in mice. Briefly, liquid Matrigel (Becton Dickinson & Co., NJ) is injected subcutaneously near the abdominal midline or the dorsal flank of the animal using a 25-gauge needle. Growth factor-reduced Matrigel supplemented with 8.3 nM basic fibroblast growth factor (bFGF, Collaborative Biomedical Products, MA) stays in liquid form at 4 °C. bFGF is a proven and potent inducer of angiogenesis. When injected into a mouse (0.5 ml/mouse), Matrigel immediately forms a readily recoverable solid gel, which is removed at various times (not exceeding 10 days) to assess neo-vessel growth around and into the gel. Test compounds are administered according to appropriate doses and schedules. Typically at a 5-day point, mice are sacrificed, overlying skin is removed and the gels are cut out retaining the peritoneal lining for support. For quantitation of angiogenesis, two methods are employed: 1. haemoglobin content in the gel is measured using the Drabkin method (Drabkin and Austin, *J. Biol Chem.* (1932) 98:719) and Drabkin reagent kit 525 (Sigma, MO); 2. the number of blood vessels invading the Matrigel is determined by microscopic analysis after the gels are fixed, embedded in paraffin, sectioned and stained.

iv) Immunohistochemistry

The anti-cancer effects of test compounds can be evaluated in mouse xenograft models (as described above) by quantitating the effects of these compounds on tumour growth, differentiation, apoptosis and angiogenesis using immunohistochemical methods.

Tumour cell proliferation, angiogenesis and tumour immune infiltrates are delineated immunohistochemically using specific antibodies (Ki-67 for proliferation, CD31 for angiogenesis and NK1.1 for NK cells and F4/80 for macrophage). Apoptosis is delineated utilising the TUNEL assay (*In Situ* Cell Death Detection kit; Boehringer Mannheim, Laval, QC). Signal generation is accomplished by peroxidase catalyzed generation of enzyme product which is visualized microscopically. Tissue histology is determined after H&E staining of separate sections.

Briefly, tumour xenografts from treated mice are isolated, fixed and paraffin embedded individually in blocks and several 5 μm sections are cut for immunostaining and TUNEL assays. One additional section is obtained for H&E staining. For all immunohistochemical labeling, prior antigen retrieval is employed to improve detection. Typically, a 3-step amplification method is used to generate signals in immunohistochemistry that consists essentially of applying a biotinylated secondary antibody that recognizes the primary antibody, followed by avidin-peroxidase incubation. The final step is enzyme reaction in stable DAB solution. Immunohistochemical sections are counterstained with hematoxylin for tissue histology. To eliminate non-specific immunostaining with mouse monoclonal antibodies applied to mouse tissues, a specific blocking step is included in the procedure. Staining patterns are documented photographically, examined by at least two independent observers and quantitated by counting a pre-determined number of cells.

The disclosure of all patents, publications, including published patent applications, and database entries referenced in this specification are specifically incorporated by reference in their entirety to the same extent as if each such individual patent, publication, and database entry were specifically and individually indicated to be incorporated by reference.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.